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# Optimization and Evaluation of *In situ* Nasal Gel of Donepezil Hydrochloride

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## Abstract

**Introduction:** The nasal route has been explored as a route of administration, due to the benefits it offers. The formulation in the form of in situ gel has been utilized for the local and systemic effect. This type of formulation first exists in sol form, but once they are administered, it undergoes gelation to form gel, and this approach can be used for successful drug delivery system. **Methods:** Thus, in the present study, formulation of in situ gel for nasal administration for donepezil hydrochloride (HCL), by the use of 32 factorial designs, to improve its nasal bioavailability, was developed by increasing its nasal retention time and arrive at an optimized formulation. The formulation was developed by the use of cold method, by incorporation of thermoreversible polymer poloxamer 407 and mucoadhesive agent tragacanth. The in situ gel was later evaluated for different parameters such as pH, gelation time gelation temperature, gel strength, drug content, mucoadhesion, viscosity, in vitro drug diffusion, and stability. **Results:** Based on results obtained, F5 formulation was found to be optimum. The concentration of 22.5% Poloxamer 407 with 0.07% tragacanth showed promising nasal drug delivery system for donepezil HCl, with enhanced residence time due to increase in viscosity and mucoadhesion characteristics. **Conclusion:** The use of in situ gel formulation thus can effectively and safely improve the nasal residence time and absorption of donepezil HCl.

**Keywords:** Factorial design, *In situ* gel, Nasal route, Poloxamer 407, Tragacanth

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## Introduction

Alzheimer's disease (AD) is one of the types of central nervous brain disease that is featured by different symptoms that cannot be ignored. The symptoms include defeat of cognitive functions (such as memory, thinking ability, and learning), obstruction in conduct of daily activities, alter intellectual functions, and it's predicted that this disease may double by the year 2040.[1] The major cause of dementia is the AD. It is characterized by degeneration of cholinergic neurons and synaptic loss that result in deficiency of cholinergic transmission and acetylcholine levels. Hence, cholinesterase inhibitors catalyze breakdown of acetylcholine in synaptic cleft that helps in enhancement of acetylcholine for the treatment of AD.[2]

Thus, to deliver drugs to the central nervous system (CNS), nasal route can be one of the non-invasive routes that overcome the blood–brain barrier (BBB). Hanson and Frey have proposed intranasal delivery as an important novel route to bypass the BBB to deliver therapeutic agent to brain.[3] Number of studies has focused on the nasal route for CNS delivery of drug.[4] This non-invasive nasal to brain delivery of drugs provides advantages over other routes of administration, with good patient compliance. In general, in market, the acetyl-cholinesterase inhibitors are available in oral dosage forms. The oral dose of these cholinesterase inhibitors in the market is once a daily tablet or capsule (5 mg or 10 mg/day) but these cholinesterase inhibitors suffer from different gastrointestinal side effects such as nausea and diarrhea muscle convulsions. There are number of cholinesterase inhibitors that have been used to improve the levels of acetylcholine in brain.[5] One of them is donepezil hydrochloride (DPZ), is reversible acetylcholine inhibitor, and helps to produce neuroprotective effect. With that, it possesses few side effects than other inhibitors, so it can be considered as first line of treatment of AD. Hence, there is a need to develop a formulation that will deliver cholinesterase inhibitors for the management of AD.[5]

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Thus, the aim of this study was to develop *in situ* nasal gel, by application of $3^2$ factorial designs that helped to characterize and reach an optimized formulation.

## Materials And Methods

Donepezil HCL was obtained gift sample from Cipla Pharmaceutical and Research Center, Patalganga, Navi Mumbai. Poloxamer 407 was purchased from Evonik Catalysts India Private Ltd., Mumbai. Tragacanth, glycerin, and benzalkonium chloride were purchased from Research Fine Lab, Mumbai.

### Methods

#### *Optimization of the in situ gels using factorial design*

On the basis of the results obtained from preliminary trials and use of Design–Expert software, $3^2$ a factorial design was constructed based on two independent variables, namely,

concentration of Poloxamer 407 ($X_1$) and concentration of tragacanth ($X_2$). Each of these independent variables was used in three concentrations 20, 22.5, and 25 for $X_1$ and 0.05, 0.07, and 0.09 for $X_2$, respectively. The dependent variables measured were percent drug release ($Y_1$) and gelling temperature ($Y_2$), as shown in Tables 1-3.

### *Preparation of nasal in situ gel*

The cold method was used for the preparation of gels. The required amount of tragacanth and drug was dissolved in distilled water, afterward, the required quantity of glycerin and benzalkonium chloride was added and followed by slow addition of Poloxamer 407 with continuous stirring at 5°C. The prepared mixture was kept at 5°C overnight to attain clear solution.

### *Evaluation of in situ nasal gels*

#### Appearance

The appearance of gel was examined for clarity. The clarity of various formulations was determined by visual inspection against black and white background.[6]

#### Determination of pH

The pH of gels was determined using calibrated pH meter. The determinations were done in triplicate and average of these determinations was taken as the pH of the gel.[7]

#### Drug content determination

One gram of gel was taken in 10 ml volumetric flask and diluted up to 10 ml with distilled water. One milliliter from this above solution was taken and again diluted to 10 ml with distilled water. After that, the absorbance of prepared solution was measured at particular wavelength using ultraviolet (UV)–visible spectrophotometer. The tests were carried out in triplicate.[8]

**Table 1:** Actual and coded value for *in situ* gel

| *Factor* | *Name* | *Unit* | *−1* | *0* | *+1* |
|---|---|---|---|---|---|
| $X_1$ | Poloxamer 407 | % w/v | 20 | 22.5 | 25 |
| $X_2$ | Tragacanth | % w/v | 0.05 | 0.07 | 0.09 |

**Table 2:** Responses for *in situ* gel

| *Response* | *Name* | *Unit* |
|---|---|---|
| $Y_1$ | % Drug release | % |
| $Y_2$ | Gelling temperature | °C |

**Table 3:** Composition and formulation codes of *in situ* gels

| *Ingredient (%)* | *F1* | *F2* | *F3* | *F4* | *F5* | *F6* | *F7* | *F8* | *F9* |
|---|---|---|---|---|---|---|---|---|---|
| Donepezil HCL | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Poloxamer 407 | 22.5 | 20 | 22.5 | 20 | 22.5 | 25 | 25 | 20 | 25 |
| Tragacanth | 0.09 | 0.07 | 0.05 | 0.05 | 0.07 | 0.09 | 0.05 | 0.09 | 0.07 |
| Glycerin | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Benzalkonium chloride | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Distilled water (q.s.) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

### Gelation Time

The sol-gel transition temperature (Tsol-gel) of the formulation was determined by test tube inversion method, by transferring 2 ml of prepared formulation to a test tube (10 ml) and sealed with paraffin. This test tube was placed in the constant temperature water bath at 37°C.[9]

### Gelation Temperature

The Tsol-gel of the formulation was determined by test tube inversion method, by transferring 2 ml of the formulation in test tube and sealed with paraffin. This test tube was placed in the constant temperature water bath. The temperature of water bath was increased in the increments of 2°C and left to equilibrate at each new temperature. However, in the region of Tsol-gel, temperature was raised slowly in the increments of 0.5°C. The formulation was examined for gelation which was said to have occurred when the meniscus of gel would no longer move on tilting at 90°.[10]

### Gel Strength

The gel strength, which is an indication for the viscosity of the nasal gel at physiological temperature, was measured by measuring the force required to settle down the gel at 35°C temperature. A sample of 20 g of nasal gel was put in 100 ml graduated cylinder and gelled in water bath at 35°C. A weight of 35 g was placed over the gelled solution. Thus, it was measured by time is seconds required by the weight to penetrate 5 cm into the nasal gel.[11]

### Mucoadhesion

The mucoadhesive potential was determined by measuring a force required to detach the gel from nasal mucosa. The mucoadhesive force [Figure 1] expressed as the detachment stress in dynes/cm$^2$ was determined from the minimal weight that detached the gel from surface of nasal mucosa.[12]

Mucoadhesive strength (dynes/cm$^2$) = mg/A

where,

m = weight required for detachment in gram

g = acceleration due to gravity

A = area of mucosa exposed

### Viscosity

The viscosity of prepared gel formulations was measured using Brookfield DV-II pro-plus viscometer. For temperature-dependent study, formulation was subjected to constant shear rate at different temperature from 26°C to 40°C. During this testing, the temperature was raised gradually by 2°C and viscosity of the sample was measured after attaining the set temperature. Measurements were done in triplicate. Steady shear sweep test was carried out by measuring the viscosity at constant temperature of 25°C and 34°C but varying the rotation speed of spindle from 5 to 100.[13]

### ***In vitro*** Diffusion Study

This study was carried out using Franz diffusion cell [Figure 2] having 2.0 cm diameter and 25 ml capacity and water jacketed which was fabricated with glass. Dialysis membrane was used as diffusion membrane. Prior conduct of experiment, pieces

of dialysis membrane were soaked in phosphate buffer having pH 6.4 for 24 h. The receptor compartment of diffusion cell was filled with phosphate buffer pH 6.4. The dialysis membrane was mounted in between donor and receptor compartment of the diffusion cell. The position of the donor compartment was adjusted so that the membrane just touches to diffusion medium. The temperature was maintained at 37°C. The content of receptor membrane was stirred using magnetic stirrer. Then, 2 ml formulation was taken in the donor compartment. At specific time interval of 1 h, 1 ml samples were withdrawn from the receptor compartment and replaced with the same volume of fresh phosphate buffer pH 6.4 after each sampling, for a period of 8 h. The samples withdrawn were filtered and analyzed by spectrophotometrically at 271 nm using UV–visible spectrophotometer.[14,15]

### Stability Study

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of variety of environmental factors such as temperature, humidity, and light to establish a retest period for a product in recommended storage conditions. Stability is the extent to which a product remains within specific limits throughout its period of storage and use. The selected formulation was stored at two different temperatures 5°C ±2°C and 40°C ±2°C temperature with 75% ±5% RH for 6 months. Samples were withdrawn and tested for pH, gel strength, drug content, viscosity, and appearance after every 1 month till 6 months.[16]

## Results And Discussion

### Appearance

All the formulations showed a clear appearance in the sol form.

### pH

The pH of all formulation was found to be in the range of 5.5 ± 0.5–5.7 ± 0.5 which is in the nasal (4.5–6.4) pH range.

### Drug Content

The percent drug content for all formulations of the drug contents was found in the range of 95.14–98.02%.

**Table 4:** ANOVA for response surface linear model

| *Source* | *Effect on % drug release* | |
|---|---|---|
| | *P-value Probe>F* | *R-squared* |
| Model | 0.0065 | 0.9968 |
| A: Poloxamer 407 | 0.0050 | |
| B: Tragacanth | 0.0116 | |

*P*<0.05 indicates that model A and B are found to be significant

**Table 5:** ANOVA for response surface linear model

| *Source* | *Effect on gelling temperature* | |
|---|---|---|
| | *P-value Probe>F* | *R-Squared* |
| Model | 0.0011 | 0.9995 |
| A: Poloxamer 407 | 0.0006 | |
| B: Tragacanth | 0.0227 | |

*P*<0.05 indicates that model A and B are found to be significant

### Gelation Temperature ($T_1$)

It is the temperature at which liquid phase gets converted into gel phase at a particular temperature as shown in [Figure 3]. Gelation temperature range suitable for nasal gel is 32–35°C. Gelation temperature for all formulations was found in the range of 25–37°C. The gelation temperature depends on the concentration of the polymer, thus as the concentration of Poloxamer 407 increases, there was decrease in gelation temperature. At gelation temperature, liquid phase makes transition into gel. Due to the addition of tragacanth and donepezil HCl, there is change in $T_1$ of gel formation. Study shows that as the concentration of tragacanth and poloxamer increases, gelation temperature decreases. This indicates that the mucoadhesive polymer has significant $T_1$ lowering effect. The gelation temperature lowering effect might be caused due to increased viscosity after dissolution of mucoadhesive polymer, as shown in Figure 3.[17]

### Gel Melting Temperature ($T_2$)

The gel melting temperature ($T_2$) was also found to increase with increase in concentration of tragacanth and Poloxamer 407. The

**Figure 1:** Measurement of mucoadhesive force of *in situ* gel

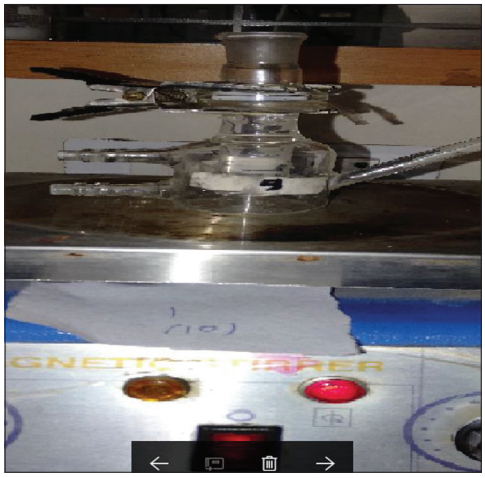

**Figure 2:** Drug diffusion study

gel melting temperature of all the formulations ranged from 42 to 62°C as depicted in [Figure 4].

## Mucoadhesive Strength

Use of polymers with strong bioadhesive capacity can significantly limit the total clearance of the formulation from the nasal cavity. An optional system for nasal drug delivery would, therefore, be fluid enough for easy administration yet would not undergo rapid initial clearance and would have sufficient interaction with the mucosal surface to limit its clearance for extended time periods. Residence time of any formulation in nasal cavity depends on the mucoadhesive strength of polymers. The result obtained clearly indicated that as the concentration of tragacanth increased, the mucoadhesive strength of gel increased. Hence, it can retain on the nasal surface for longer period of time. Formulations F1, F5, F6, F7, and F9 were found to have sufficient mucoadhesive strength [Figure 5].[18]

## Gel Strength [Figure 6]

The gel strength was found to be affected by concentrations of gelling and bioadhesive polymers. *In situ* gel must have suitable gel strength so as to administer easily and that can be retained at nasal mucosa without leakage after administration. In the gels, tragacanth and Poloxamer 407 were found to increase the gel strength with their increasing concentrations. The gel strength value between 25 s and 50 s was considered sufficient. The gel strength <25 s may not retain its integrity and may erode rapidly while gels having strength >50 s are too stiff and may cause discomfort to the mucosal surfaces.[19]

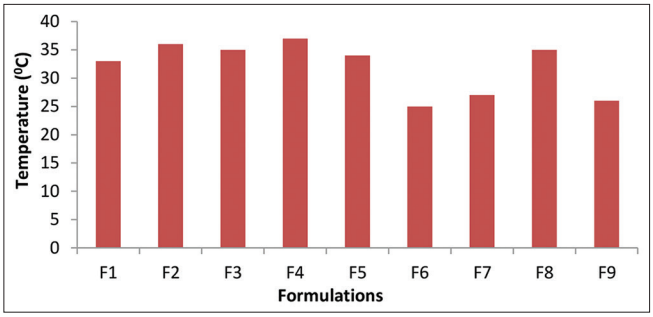


**Figure 3:** Gelation temperature of formulated batches

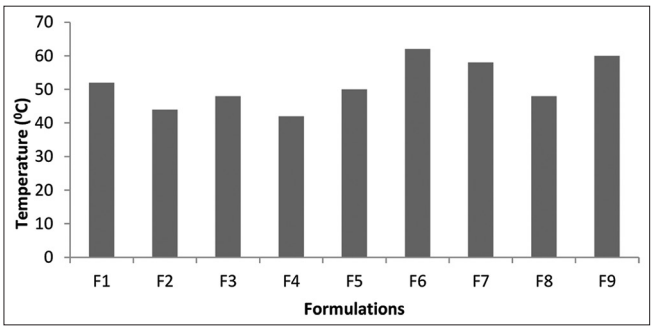


**Figure 4:** Gel melting temperature of formulated batches

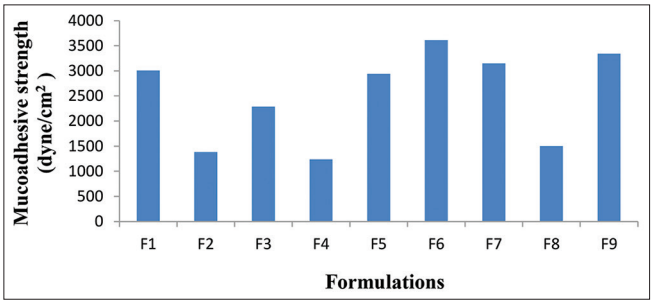


**Figure 5:** Mucoadhesive strength of formulated batches

## Gelation Time

It was found to be <1 min.

## Rheological Analysis

The viscosity determination of formulation from F1 to F9 was also carried out at different temperatures from 26°C to 40°C [Figure 7]. The viscosity values of formulation (F1 to F9) were measured at different share rates [Figure 8, 9] (5–100 rpm) at constant temperature (25°C and 34°C). The viscosity determination of formulation from F1 to F9 was also carried out at different temperatures from 26°C to 40°C.

From the above graph, it is clear that as the temperature increases, the viscosity of the formulation increases. At the gelation temperature, there are sudden increases in the viscosity indicating the conversion of sol to gel. It was found that the rheological parameter was directly dependent on the polymer concentration of the formulations. This indicated the conversion of these formulations from sol to gel. It was also observed that the viscosity of all the formulations was decreasing with increase in shear rate.

The permeability study showed that the drug permeation decreased with an increase in tragacanth and Poloxamer 407 concentrations. The retarding effect of mucoadhesive polymers, tragacanth could be attributed to their ability to increase overall product viscosity and gel matrix structure. The result clearly showed that tragacanth and Poloxamer 407 affected the drug release. The % drug release of optimized batch (F5) was found to be 94.1% ±0.3% [Figure 10]. The ANOVA analysis for drug release is as shown in Table 4.

## Optimization Study

### *Effect of Poloxamer 407 and tragacanth on % drug release*

The drug permeation decrease with an increase in tragacanth and poloxamer 407 concentrations. The retarding effect of mucoadhesive polymers, tragacanth could be attributed to their ability to increase overall product viscosity and gel matrix structure. The result clearly showed that tragacanth and poloxamer 407 affected the drug release.

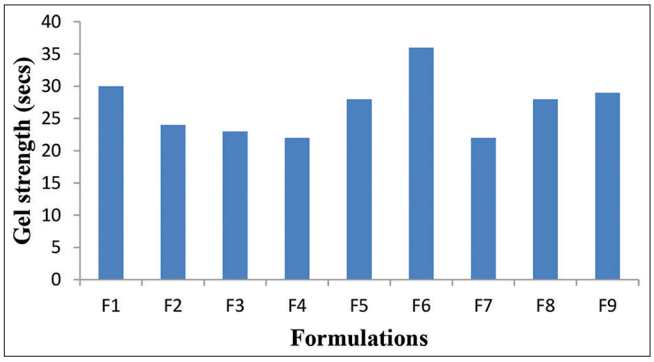


**Figure 6:** Gel strength of formulated batches

*Final equation in terms of coded factors*

% Drug release = +88.31 + 0.2184*A+5.28*A+3.38*B+0.0449*B

The graph [Figure 11] reveals the contribution of Poloxamer 407 and tragacanth to percent drug release. As the signs of Poloxamer 407 and tragacanth both are positive, it is concluded that both polymers have direct relation with viscosity. The independent and response variables were related using polynomial equation with statistical analysis through Design Expert® software. The values of the coefficient $X_1$ and $X_2$ are related to the effect of these variables on the response. A positive sign of coefficient indicated a synergistic effect while a negative term indicated an antagonistic effect on the response. The lesser coefficient meant that the independent variable has more potent influence on the response. The ANOVA analysis for drug release is as shown in Table 5.

*Effect on gelling temperature*

Micelle formation is important for gel formation. And for this, temperature plays major role in the formation of micelles. The mucoadhesive polymer did had effect on the gelling temperature.

*Final equation in terms of coded factors*

Gelling temperature: = +32.17 + 4.02A*+1.95A*+1.01B*+0.2143B*

The graph [Figure 12] revealed the contribution of Poloxamer 407 and tragacanth to gelling temperature, the equation stated that Poloxamer 407 had significantly contributed to gelling temperature, tragacanth also contributed but to a lesser extent. The positive signs of Poloxamer 407 and tragacanth both conclude that both have direct relation with the gelling temperature.

## Stability Study

Studies were carried out after storing the optimized formulation (F5) at two different temperatures 5°C ±3°C and 40°C ±2°C temperature with 75% ±5% RH for 6 months. Tables 6 and 7 show the data for appearance, dug content, viscosity, gelation time, and gelation temperature. The formulation F5 showed no change in appearance after at 5°C ±3°C temperature conditions. The drug content decreased from 88.8% to 88.1% after 6 months, the viscosity of the formulation was changed. For storage condition of 25°C ±2°C temperature with 75% ±5% RH, there was also no change in the appearance, gelation temperature, and gelation time after 6 months. The drug content

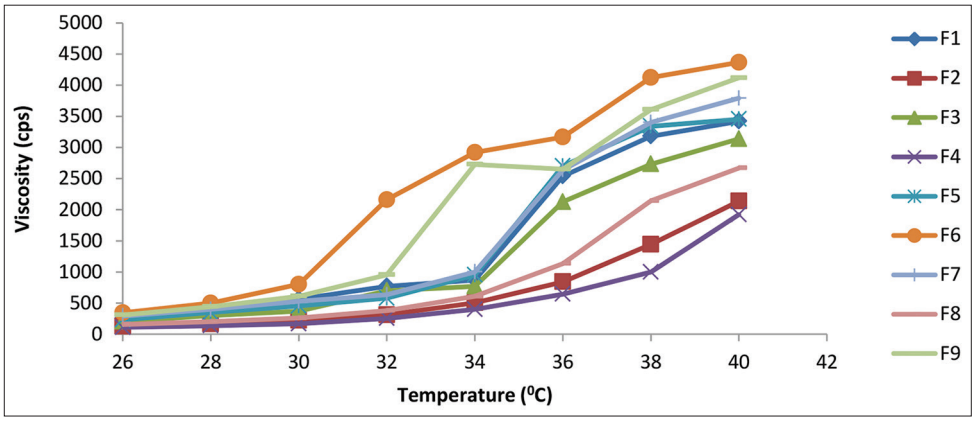


**Figure 7:** Comparison of viscosity change with temperature for formulated batches

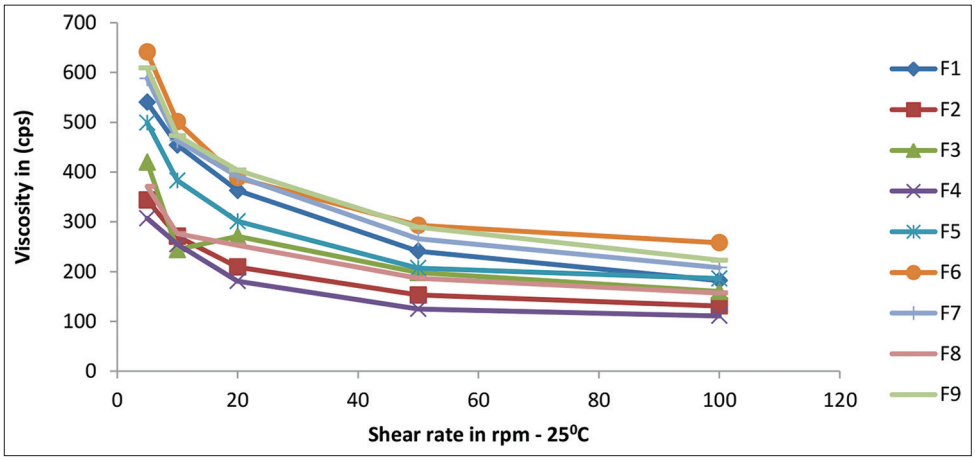


**Figure 8:** Comparison of viscosity change with change in shear rate (at 25°C)

**Table 6:** Optimized F5 formulation for stability study at 5°C±3°C

| *Time period* | *Appearance* | *% drug content* | *Viscosity (Cps)* | *Gelation time (Min)* | *Gelation temperature (°C)* |
|---|---|---|---|---|---|
| Initial | Clear | 88.8±0.5 | 1391±1.8 | <1 | 34 |
| After 6 months | Clear | 88.1±0.5 | 1375±2.1 | <1 | 34 |

**Table 7:** Optimized F5 formulation for stability study at 40°C ± 2°C (75%±5% RH)

| *Time period* | *Appearance* | *% drug content* | *Viscosity (Cps)* | *Gelation time (Min)* | *Gelation temperature (°C)* |
|---|---|---|---|---|---|
| Initial | Clear | 88.8±0.5 | 1391±3.2 | <1 | 34 |
| After 6 months | Clear | 87.6±0.5 | 1370±3.1 | <1 | 34 |

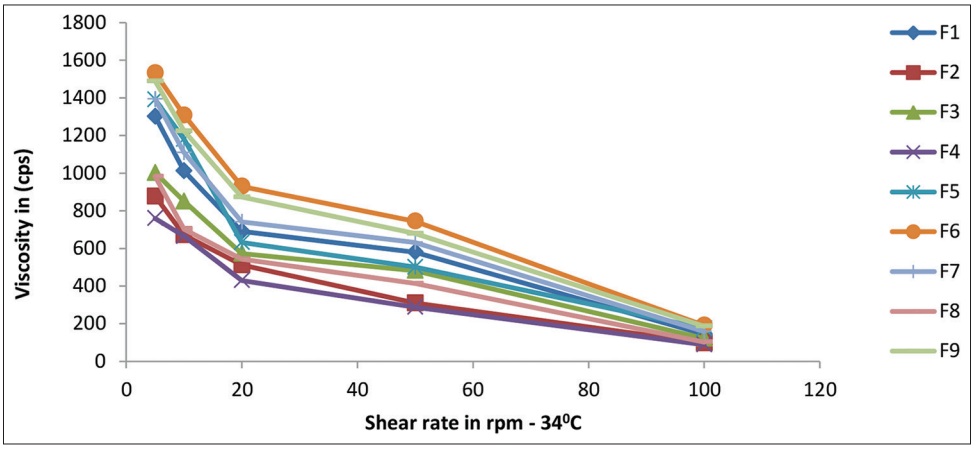


**Figure 9:** Comparison of viscosity change with change in shear rate (at 34°C)

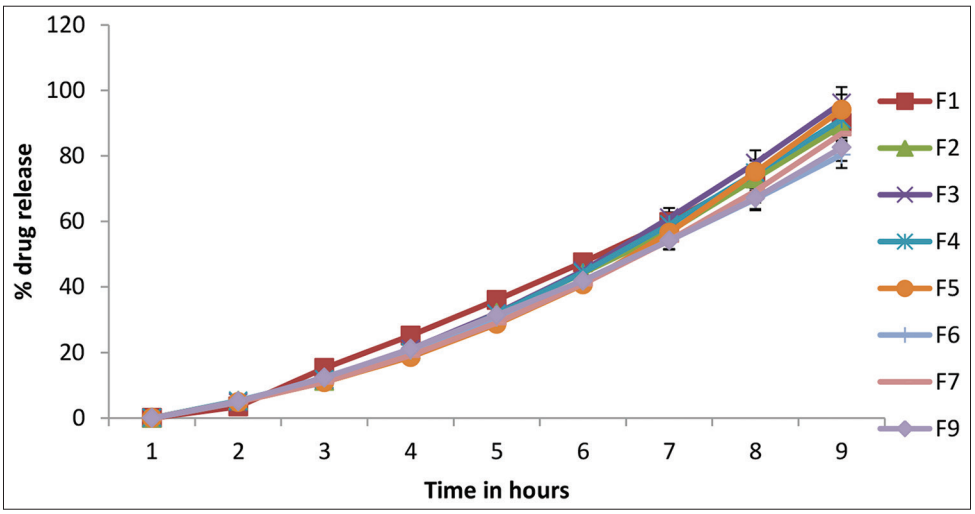


**Figure 10:** The drug release features of formulated batches

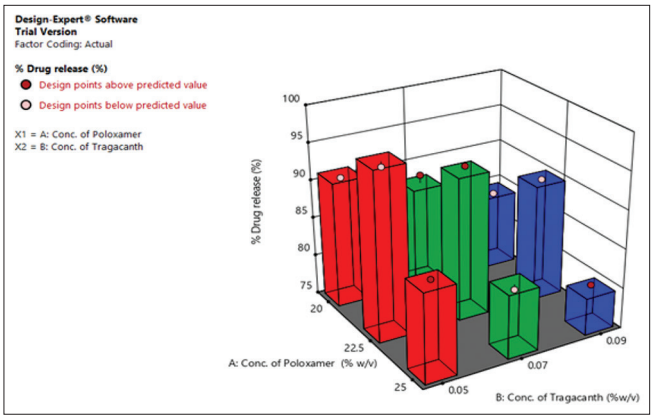


**Figure 11:** Three-dimensional surfaces plot showing the influences of Poloxamer 407 and tragacanth on % drug release

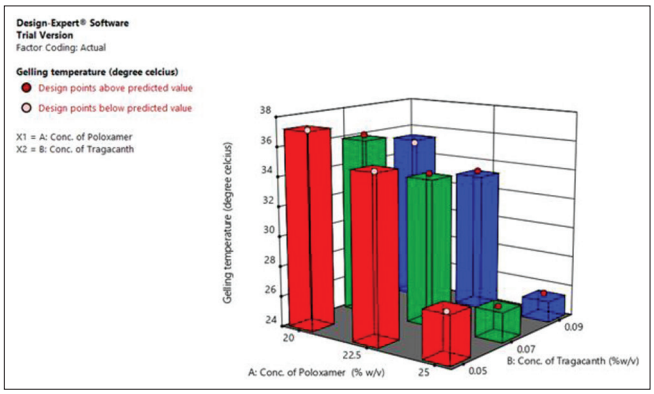


**Figure 12:** Three-dimensional surfaces plot showing the influences of Poloxamer 407 and tragacanth on gelling temperature

decreased from 88.8% to 87.6% after 6 months. Furthermore, the viscosity of the formulation was changed. The results depicted a slight change in the evaluation parameters, thus, the formulation F% was found to be stable as per the guidelines.

## Summary And Conclusion

The study can be concluded in formulation of successful in situ gel for nasal administration for an Alzheimer's drug. Based on results obtained, F5 formulation was found to be the optimized that consisted of 22.5% Poloxamer 407 with 0.07% tragacanth that enhanced nasal residence time due to increase viscosity and mucoadhesion characteristics, with good permeation enhancement. The study demonstrated that the use of in situ thermogelling agent Poloxamer 407 and mucoadhesive polymer tragacanth could effectively and safely improve the nasal residence time and absorption of DPZ.

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