Formulation and Evaluation of Nasal in situ Gel for Alzheimer Disease.


Department of Pharmaceutics, Ashokrao Mane College of Pharmacy, Peth - Vadgaon.
Maharashtra, India.

The aim of present study was to formulate and develop in situ gelling system for nasal administration for an anti-Alzheimer’s drug. The objective of this research work was to improve the nasal bioavailability of drug by increasing its nasal retention time and checked the effect of temperature on the preparation of formulation. The Donepezil was used as model drug. Formulation was developed to reduce the mucociliary clearance by using mucoadhesive polymer in gel, thereby increasing the contact time with nasal mucosa and hence improving the absorption of drug. The in-situ gelation was achieved by the use of Pluronic F127, which exhibits thermo reversible gelation property and carbopol 974P was used as the mucoadhesive agent. Gel was prepared at different temperature 50°C, 150°C, 250°C. The prepared in-situ gel was evaluated for pH, gelation temperature, drug content, mucoadhesive force, gel strength and viscosity measurement. Further in-situ gel was evaluated for its in vitro drug diffusion study.

Keywords: Anti- Alzheimer’s drug, in situ gel, Nasal drug absorption, in-vitro diffusion study.
INTRODUCTION

Now days, most of the people like to stay healthy by using correct diet and fitness. They try to do anything to prevent diseases which happen more often with increases age [1]. After age 40, most of peoples begin to worry about losing their memories and becoming demented. This is true once reaching their fifties, because there has been an increase in the incidence of neurodegenerative diseases such as Alzheimer’s disease (AD), Parkinson’s disease and Lou Gehrig’s disease over the last two decades [2]. About 33.9 million people worldwide have AD at present, and according to estimates from the Alzheimer’s Association. One in eight older Americans has Alzheimer’s disease. Alzheimer’s disease is the sixth-leading cause of death in the United States. All available treatment is for relatively small symptomatic benefit but remain palliative in nature. It may be inconvenient for the patients, when there is long term therapy of medication. The most of patients with Alzheimer’s disease are older, so, it is not possible to give drugs orally. The nasal mucosa is one of the most permeable and highly vascularized site for drug administration ensuring rapid absorption and onset of therapeutic action. After oral administration of drug, it is difficult to concentrate the required amount of drug in brain due to extensive first-pass metabolism and also causes side effects like gastric irritation, nausea and vomiting etc. As compliance for better therapy of Alzheimer’s disease, intranasal delivery can be utilized as key for bypassing presystemic metabolism of drug and adverse effects caused due to oral administration. So, for improving efficacy and reducing side effects, in situ nasal gel is prepared. It gives prolong residence time of the dosage form at the site of application or absorption and facilitate an intimate contact of the dosage form with the underlie absorption surface and thus contribute to improved and / or better therapeutic performance of the drug. The reduction of post-nasal dripping due to its high viscosity, reduction of the taste impact due to reduced swallowing, reduction of anterior leakage of the formulation, reduction of irritation by using soothing/emollient excipients, and target delivery to the mucosa for better absorption.

Types of Alzheimer Disease

There are mainly two types of Alzheimer disease. First is Early-onset Familial and second is Late-onset Sporadic.

Early-onset Familial Alzheimer disease

In some cases where only 10% of all persons diagnosed with Alzheimer disease develop symptoms before the age of 65 years. They are said to have early-onset Alzheimer disease, and approximately 10% of these early-onset cases have a familial form of the condition, which is transmitted as an autosomal dominant trait. Mutations in three genes amyloid precursor protein, presenilin-1 and presenilin-2 cause the majority of cases of familial Alzheimer disease.

Late-onset Sporadic Alzheimer disease

Generally Alzheimer is diagnosed after the age of 65 years, when it is referred to as late-onset Alzheimer disease. The condition affects 5% of the population aged over 65 years and more than 20% of the population over 85 years [3].

Signs and Symptoms of Alzheimer’s disease [4.5.6]

1. Memory loss that disrupts daily life
2. Challenges in planning or solving problems
3. Difficulty completing familiar tasks at home, at work or at leisure
4. Confusion with time or place
5. Trouble understanding visual images and spatial relationships
6. New problems with words in speaking or writing
7. Misplacing things and losing the ability to retrace steps
8. Decreased or poor judgment
9. Withdrawal from work or social activities
10. Changes in mood and personality.

Pathology
Neocortex is important, which is responsible for processing the sensory information relayed to the brain, controlling voluntary movements, performing conscious thought and other mental activities. The Limbic system is mainly responsible for emotions and instinctive behavior. The hippocampus plays important roles in learning and short-term memory. During AD, neurons of some brain areas, especially neocortex and limbic system includes hippocampus, amygdala and their associated cortices, gradually deteriorate and undergo death. A definite diagnosis of Alzheimer disease can be made only by autopsy examination of a patient’s brain. This neuropathological evaluation reveals gross cerebral atrophy, signifying loss of neurons and synapses in the cerebral cortex and certain subcortical regions [3,7,8].

The Alzheimer’s brain is markedly smaller in size. Folds and grooves of outer layers are atrophied and ventricles are larger. By comparison, the healthy brain suffers only modest loss of mass during aging. The reductions in the size of specific brain regions in people with AD as they progressed from mild cognitive impairment to Alzheimer’s disease and in comparison with similar images from healthy older adults [9].

Figure 1: The Healthy brain (Left side) and Alzheimer’s brain (Right Side) [6,9].

Nasal Drug Absorptions
Mechanism of Drug Absorptions
The very important and first step in absorption is drug must pass through the mucus layer. From this layer small unchanged drugs/particles pass easily but large, charged drugs are more difficult to cross it. (figure 2) Mucin is the
principle protein of mucus, which has tendency to bind to solute, hindering diffusion. Additionally, the structural changes in the mucus layer are possible as a result of environmental changes (i.e. pH, temperature, etc.). There are so many absorption mechanisms through the mucosa have been proposed but only two mechanisms have been considered predominantly. One of that is fast rate and lipophilicity dependant and other is slower rate and sensitive to variation in molecular weight [10, 11].

![Figure 2: Mechanism of drug absorption](image)


First mechanism - This mechanism involves an aqueous route of transport, which is also known as the paracellular route [10, 11].

Second mechanism - this mechanism involves transport through a lipoidal route that is also known as the transcellular process [10, 11].

Advantages of Nasal Drug Delivery System
The nasal drug delivery has many advantages over other routes. Such as;
1. Easy to administration, non-invasive, rapid and comfortable [12, 13].
2. Easy accessibility to blood capillaries [12, 13]
3. Avoid side effects like nausea and vomiting which is normally seen after oral administration [14].
4. Avoids destruction in the gastrointestinal tract (chemical and enzymatic degradation of drugs), hepatic “first pass” elimination and gut wall metabolism allowing increased, thus potential for dose reduction compared to oral delivery, results in reduction in side effects [15, 16, 10].
5. Drugs which are orally not absorbed or unstable in gastric fluids can be delivered to the systemic circulation by nasal drug delivery [17, 15].
6. The nasal route is an alternate to parenteral route, especially for proteins & Peptides.
7. Convenient for the patients, when compared with long term therapy of parenteral medication [18].
8. Good penetration of, lipophilic, low molecular weight drugs through the nasal mucosa [18, 19].
9. Rapid absorption and fast onset of action due to relatively large absorption surface and high vascularization [18].
10. Bioavailability of larger drug molecules can be improved by means of absorption enhancer or other approach [18, 19, 20].
11. Lesser opportunity for drug-drug interaction potential.
12. Rapid attainment of peak levels for certain category of drugs is observed [18].
13. For direct delivery of drug to the central nervous system via the olfactory region, thus by-passing the blood brain barrier [16].
14. Direct delivery of vaccine to lymphatic tissue and induction of a secretory immune response at distant mucosal site [16].
15. It facilitates the treatment of many neurologic and psychiatric disorders [21].

Materials
Donepezil and Carbopol 974P was obtained gift sample from Gen Pharma Intl. Pvt. Ltd. Bhosari. Pluronic F-127 was purchased from BASF India Ltd. Turbhe (Mumbai). Glycerine and Sodium hydroxide purchased from Loba Chemicals, Mumbai. Sodium metabisulphite, Benzalkonium chloride, Potassium dihydrogen phosphate and Methanol was purchased from Molychem Pvt. Ltd., Mumbai.

Formulation of in situ nasal gel
As per runs obtained in design table 1, total nine batches were prepared. Firstly, the Carbopol 974P was added in some amount of distilled water with continuous stirring or trituration. Then 18% w/w Pluronic F-127 (Poloxomer 407) added and triturate until solution was formed. The drug along with other additives like Glycerin, Benzalkonium chloride, Sodium metabisulphite were dissolved separately as per concentration mentioned in table 3 and added to polymer solution with constant stirring. This resulting formulation was then kept overnight at 4-5°C until clear liquid solution was formed to ensure complete dissolution.

Experimental Design
In the present work factorial design was used study to formulate and develop effective, functional and feasible nasal in-situ gel for its sustained release effect. A $3^2$ full factorial design was used to process variables that were thought to affect the release of Donepezil from in situ gel.

<p>| Table 1: $3^2$ full factorial design |</p>
<table>
<thead>
<tr>
<th>Batch code</th>
<th>$X_1$</th>
<th>$X_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td>F2</td>
<td>-1</td>
<td>0</td>
</tr>
<tr>
<td>F3</td>
<td>-1</td>
<td>+1</td>
</tr>
<tr>
<td>F4</td>
<td>0</td>
<td>-1</td>
</tr>
<tr>
<td>F5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F6</td>
<td>0</td>
<td>+1</td>
</tr>
<tr>
<td>F7</td>
<td>+1</td>
<td>-1</td>
</tr>
<tr>
<td>F8</td>
<td>+1</td>
<td>0</td>
</tr>
<tr>
<td>F9</td>
<td>+1</td>
<td>+1</td>
</tr>
</tbody>
</table>
Table 2: $3^2$ Independent variables with their levels.

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(-1)</td>
</tr>
<tr>
<td>X1</td>
<td>0.5</td>
</tr>
<tr>
<td>X2</td>
<td>5</td>
</tr>
</tbody>
</table>

Where, the independent variables were:

$X_1$ = Amount of Carbopol 974P (% w/w)

$X_2$ = Temperature ($^\circ$C)

The empirical second order equation of $3^2$ full factorial design:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1^2 + \beta_{12} X_2^2$$

$Y$ = Dependent variable

$\beta_0$ = intercept

$\beta_1$, $\beta_2$, $\beta_{12}$ = The coefficients from the response of the formulation in design

The different ingredients used in the formulation with its concentration are enlisted below;

Table 3: Formulation parameter of preparation of in-situ gel

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Name of Ingredients</th>
<th>Concentration (%w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Donepezil Hydrochloride</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Pluronic F-127</td>
<td>18</td>
</tr>
<tr>
<td>3</td>
<td>Carbopol 974P</td>
<td>0.5-1.5</td>
</tr>
<tr>
<td>4</td>
<td>Glycerine</td>
<td>0.5</td>
</tr>
<tr>
<td>5</td>
<td>Sodium Metabisulphite</td>
<td>0.25</td>
</tr>
<tr>
<td>6</td>
<td>Benzalkonium Chloride</td>
<td>0.02</td>
</tr>
<tr>
<td>7</td>
<td>Distilled water (q.s.)</td>
<td>100</td>
</tr>
<tr>
<td>8</td>
<td>Temperature ($^\circ$C)</td>
<td>5-25</td>
</tr>
</tbody>
</table>

Evaluation of Nasal Gel

Measurement of pH of Formulation

1ml quantity of each formulation was transferred to a beaker and diluted by using distilled water to make 25ml. pH of the resulting solution was determined using digital pH meter (Elico LI 615 pH meter) $^{22,23,24}$.

Measurement of Gelation Temperature

It is determined by using method described by Miller and Donovan technique. According to this method, 2 ml sample of gel was transferred to a test tube. Then test tube was immersed in a water bath. The temperature of water bath was increased slowly and examined gelation by tilting the test tube. At certain point the meniscus would no longer move upon tilting the test tube to 900, recorded the temperature at that point, which was said as gelation temperature of that sample $^{23,24,25}$. 
Drug Content Estimation
1ml of formulation was taken in 50 ml volumetric flask, diluted with distilled water and volume adjusted to 50 ml. One ml quantity from this solution was again diluted with 10 ml of distilled water. Finally the absorbance of prepared solution was measured at 313.0 nm by using Shimadzu-1800 UV visible spectrophotometer \[23,24\].

Viscosity Measurement
The viscosity measurements were carried out by using Brookfield DV-III Ultra Programmable Rheometer. The LV-4 i.e. spindle no. 64 was used and rotated at 0.2 rpm. The temperature of sample was maintained with the help of temperature control unit. The measurement was taken at 37°C \[23,25,26\].

Gel strength determination
This test was performed by using „Gel strength apparatus“ . A 50 ml sample was placed in 100 ml graduated measuring cylinder and placed into water bath at 37°C. The sample was converted into gel. Then marking at upper meniscus level was done as a starting point and measured 5 cm distance from that point and marked as a end point. Stopwatch was kept ready. Then piston was placed onto the gel which having weight 35 g and measure the time in seconds which required for moving the piston 5 cm down through the gel. It is indication for the viscosity of the nasal gel at physiological temperature \[24, 25\].

Determination of Mucoadhesive Force
The mucoadhesive strength of each nasal in situ gel formulation was determined by measuring the force required to detach the formulation from sheep nasal mucosal tissue. This determination was done by using mucoadhesive measuring device modified in laboratory (figure 6.2) according to previously reported methods using tissue specimen obtained from the mucosal side of sheep nasal cavity obtained from local slaughter house. From pieces of tissue of nasal mucosa, a section was cut and the mucosal side was instantly fixed towards the upper side of each glass vial using a rubber band by keeping mucosal side out. The diameter of each exposed mucosal membrane was 2 cm. The vials with nasal mucosa were stored at 37°C for 5 minutes. Then one vial with a section of mucosa was connected to the balance in inverted position and another vial was placed on a height adjustable pan. The constant amount of gel sample (0.5 ml) of each formulation was applied onto the nasal mucosa of first vial. After that the height of second vial was adjusted so, mucosal surfaces of both vials come in intimate contact. Immediately a force was applied for two minutes to ensure intimate contact between tissues and the samples. The upper vial was moved upwards at a constant force, while it was connected to the balance. Weights were added at a constant rate to the pan on the other side of the modified balance. The weights was added until the two vials got detached. The mucoadhesive force expressed as the detachment stress in dyne/cm², was determined from the minimal weights that detached or separated the tissues from the surface for each formulation using the following equation,

\[
\text{Detachment stress (dyne/cm}^2\) = m \times g / A
\]

Where,

\[
m = \text{Weight required for detachment of two vials in gm} \]
\[
g = \text{Acceleration due to gravity [980cm/s]} \]
\[
A = \text{Area of tissue exposed, which is equal to } \pi r^2.
\]
The nasal mucosa was changed for each measurement. Measurements were repeated three times for each of the gel preparations [27, 22].

Figure 3: Modified balance for mucoadhesion study [27, 28].

In-vitro Diffusion study:
In vitro diffusion study is one of the important criteria for mucoadhesive in situ gel. This study was carried out by using Franz diffusion cell having 2.0 cm diameter and 25 ml capacity and water jacketed which was fabricated with glass. For this study, dialysis membrane 110 LA 395-1 MP (HiMedia laboratories Pvt. Ltd. Mumbai) was used as diffusion membrane. Before starting the experiment, these pieces of dialysis membrane were soaked in phosphate buffer having pH 6.4 for 24hrs. 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.

Table 4: Parameter for In-vitro Diffusion study.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>In-vitro Diffusion Apparatus</td>
<td>Franz Diffusion Cell</td>
</tr>
<tr>
<td>Diffusion Membrane</td>
<td>Dialysis Membrane 110 LA 395-1 MP</td>
</tr>
<tr>
<td>Diffusion Medium</td>
<td>Phosphate Buffer pH 6.4</td>
</tr>
<tr>
<td>Volume of Diffusion Medium</td>
<td>25.0 ml</td>
</tr>
<tr>
<td>Volume of Sample size</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Temperature</td>
<td>37 °C</td>
</tr>
<tr>
<td>Time interval</td>
<td>8 hrs.</td>
</tr>
</tbody>
</table>

The temperature was maintained at 37°C by transferring the hot water through water jacket. The content of receptor membrane was stirred by using magnetic stirrer. Then 2 ml formulation was taken in the donor
RESULTS AND DISCUSSION

Preformulation Study
Characterization of Donepezil
Melting Point Determination
The melting point of Donepezil hydrochloride was found to be 223°C. The melting point values reported for Donepezil hydrochloride in the range of 223°C to 227°C.
Calibration Curve of Drug
The wavelength of maximum absorbance (λ max) selected was 313 nm for Phosphate Buffer having pH 6.4 for Donepezil hydrochloride. The graph which was plotted was found to be linear in the concentration range of 0 to 100 μg/ml and obeys the Beer-Lambert’s law in the same ranges. It is shown in figure 7.

![Figure 4: calibration curve of Donepezil hydrochloride](image)

Fourier Transform Infrared Analysis

![Figure 5: FT-IR spectrum of Donepezil.](image)
The IR spectrum of Donepezil hydrochloride obtained on Cary 630 model of Agilent technology presented in Figure. results shows the presence of characteristic peaks such as Mono-substituted benzene, Aromatic hydrocarbons, C=C Stretching of aromatic ring, N-H Bending shows at 749, 1500, 1589, 1605 respectively.

Drug Excipients Compatibility Study

Optimization
Selection of Concentration of Pluronic F127
Generally, temperature range of the nasal mucosa lies in between 32°C to 37°C. Hence, the suitable ranges of gelation temperatures have been considered in between 32°C to 35°C. A gel might be formed leading to difficulty in manufacturing, handling, and administering, if the gelation temperature of a thermoreversible formulation is lower than 30°C and if the gelation temperature of thermoreversible gel is higher than 37°C, then the formulation remains in liquid form still exists at nasal mucosal temperature, which results in the nasal clearance of the administered drugs before absorptions and causes loss in formulation and drugs.

Table 5: Selection of concentration of Pluronic F-127

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>Concentration of Pluronic F-127 (% w/w)</th>
<th>Gelation Temperature (°C)Mean ± S.D. n=3</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>16%</td>
<td>44.7±0.6</td>
</tr>
<tr>
<td>P2</td>
<td>18%</td>
<td>34.3±1.2</td>
</tr>
<tr>
<td>P3</td>
<td>20%</td>
<td>27.7±0.6</td>
</tr>
</tbody>
</table>

The temperature of the nasal cavity is 37°C, so, this study aimed that preparing the liquid formulations of pluronic F-127 which may gels below 37°C. The gelation of pluronic F-127 vehicles was known to result from the change in micellar number with temperature. Formation of number of micelles increases with increase in temperature, which results the negative coefficient of solubility of block copolymer micelles. Eventually the micelles become so tightly packed that the solution becomes immobile and gel is formed.
Conformational changes in the orientation of the methyl groups in the side chains of poly (oxypropylene) polymer chains, constituting the core of the micelle, with expulsion of the hydrating water from the micelles will contribute to the gelation phenomenon. For selection of concentration of Pluronic F-127 its gelation temperatures were observed at the concentration 16%, 18% and 20% w/v and it was found that the gelation temperature decreased with increasing its concentration. It may be attributed to the higher number and volume occupied by micelles at low temperature. After increasing concentration of pluronic F-127, the gel structure becomes more closely packed with the arrangement in the lattice pattern. From table 10 it was found that only 18% concentration of pluronic F-127 gel showed ability to form gel in the range of 31°C to 35°C. So, 18% w/v concentration of PF127 was used for further studies.

**Evaluation of In Situ Gel Formulations**

**pH**

The pH of all formulations was found to be in the range of 5.2-5.9, which is well within the range specified for nasal formulation. Because, lysozyme was present in the nasal secretions, which was responsible for destroying certain microbes at acidic pH. Under alkaline pH lysozyme is inactive and nasal tissue is susceptible to microbial infection. Hence, it was advisable to keep the formulation’s pH in the range of 4.5-6.5. (Table 7)

**Gelation Temperature**

The gelation temperatures of all batches were determined by visual method. As concentration of mucoadhesive polymer Carbopol 927P in formulation increases from 0.5% to 1.5% there were decreases in gelation temperature. (Table 7, figure 7). The temperature condition during formulation preparation increases from 5°C to 25°C there were decrease in gelation temperature. The gelation temperature-lowering effect of mucoadhesive polymers might be caused in part by the increased viscosity after dissolution of mucoadhesive polymers and due to increment in formulation temperature there may be number of micelle formation increases and caused increase in viscosity.

![Figure 7: Gelation temperature of all formulation batches.](image)

The formulations containing concentration of Carbopol 974P higher than 1.5% at formulation preparation temperature 5°C, 1.0 and 1.5% at formulation preparation temperature 15°C and 0.5, 1.0, 1.5% at formulation preparation temperature 25°C were found to have gelation temperature less than 30°C, and so
were difficult to administer into the nostril. But formulations containing Carbopol 974P concentration 0.5% and 1.0% at formulation preparation temperature 5°C and 0.5% at formulation preparation temperature 15°C gelled at temperature ranging from 30°C to 35°C. These temperatures seemed to be proper for in situ gelling of the various vehicles at the nasal cavity, minimizing the loss of administered drug caused by clearance from the site of application.

Drug Content
The percentage drug content of all prepared nasal formulations were checked and found to be in the range of 98 – 100.0%

Viscosity Measurement
Table 7 shows the single apparent viscosity values, measured by using Brookfield’s DV-III Ultra Programmable Rheometer with spindle no.64 at 0.2 rpm for formulation F1 to F9. There was considerable change in viscosity at the point of gelation temperature. The viscosity was directly dependent on the polymeric content of the formulations. It is to be noted that the addition of increasing concentrations of Carbopol 974P from 0.5% to 1.5% and also by increasing formulation preparation temperature from 5°C to 25°C increases the viscosity of formulations. From viscosity studies it was found that viscosity increase with increase in concentration of pluronic F127 and with concentration of mucohesive polymer carbopol 974P and also with increase formulation preparation temperature. The relationship between viscosity and temperature may be expressed as by Arrhenius equation. Explanation of Arrhenius for relation between viscosity and temperature is not obeyed for these formulation i.e. with increase in temperature, viscosity decreases accordingly Ev decreases due to bond breakage.

Table 7: pH, gelation temperature, %drug content, viscosity, gel strength and mucoadhesive strength of all formulations batches.

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>pH</th>
<th>Gelation temperature (°C) ± S.D. n=3</th>
<th>% Drug Content</th>
<th>Viscosity (cP) at 37°C ± S.D. n=3</th>
<th>Gel Strength (sec.) ± S.D. n=3</th>
<th>Mucoadhesive strength (dyne/cm²) ± S.D. n=3</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>5.9</td>
<td>35.0±1.0</td>
<td>100.0</td>
<td>15500</td>
<td>42±1.5</td>
<td>3524.36±11.92</td>
</tr>
<tr>
<td>F2</td>
<td>5.4</td>
<td>33.7±1.1</td>
<td>99.15</td>
<td>19800</td>
<td>48±1.5</td>
<td>3857.29±16.23</td>
</tr>
<tr>
<td>F3</td>
<td>5.3</td>
<td>28.3±1.2</td>
<td>98.40</td>
<td>25100</td>
<td>60±2.5</td>
<td>4603.77±19.13</td>
</tr>
<tr>
<td>F4</td>
<td>5.8</td>
<td>31.0±1.0</td>
<td>99.15</td>
<td>17900</td>
<td>46±1.5</td>
<td>3623.20±19.63</td>
</tr>
<tr>
<td>F5</td>
<td>5.6</td>
<td>28.3±0.6</td>
<td>98.40</td>
<td>22000</td>
<td>50±2.0</td>
<td>4010.74±15.62</td>
</tr>
<tr>
<td>F6</td>
<td>5.2</td>
<td>25.3±0.6</td>
<td>98.0</td>
<td>32300</td>
<td>63±1.5</td>
<td>4853.47±07.84</td>
</tr>
<tr>
<td>F7</td>
<td>5.6</td>
<td>26.0±1.0</td>
<td>98.40</td>
<td>18700</td>
<td>50±1.5</td>
<td>3844.28±11.42</td>
</tr>
<tr>
<td>F8</td>
<td>5.6</td>
<td>24.0±1.0</td>
<td>99.15</td>
<td>23600</td>
<td>67±1.5</td>
<td>4348.87±16.24</td>
</tr>
<tr>
<td>F9</td>
<td>5.4</td>
<td>22.3±0.6</td>
<td>98.40</td>
<td>34200</td>
<td>72±2.5</td>
<td>5087.56±15.62</td>
</tr>
</tbody>
</table>

But here for thermoreversible nasal gel it was observed that with increase in temperature, viscosity increases
due to change in micellar properties of pluronic and energy of activation increases (Figure 8).

![Figure 8: Viscosity of all formulation batches.](image)

**Gel strength**

The gel strength value between 25 to 50 seconds is considered sufficient. The formulation with gel strength less than 25 second may not preserve its integrity and may erode rapidly. In case of formulations with gel strength more than 50 second is too stiff and may cause discomfort. It was observed from data, only F1, F2, F4, F5 and F7 were found to be in this range. In situ gel must have suitable gel strength so as to be administered easily and can be retained at nasal mucosa without leakage after administration. It is very important that the nasal gel formulation must have suitable gel strength. Table 7 & figure 9 showed the data of gel strength measurement.

![Figure 9: Gel strength of all formulation batches.](image)
Mucoadhesive strength
The mucoadhesive strength is an important parameter for in situ gelling nasal formulations since it prolongs the nasal clearance of gels and increases its residence time in nasal cavity prevents the gelled solution dropping out of the nose.

![Image](image.png)

**Figure 10: Mucoadhesive strength of all formulation batches.**
It means the force with which nasal gel binds to nasal mucous membranes and it is an important physiological parameter for gelling the nasal gel. It was found that, two minutes of contact time was sufficient for optimum mucoadhesive strength. If contact time increase further did not affect the mucoadhesive strength, but if contact time decreases resulted in less mucoadhesive strength resulting from insufficient time for entanglement of polymer chains with mucin. Mucoadhesive strength was determined by using detachment stress. The formulation of pluronic F-127 have adhesive properties which increased by addition of Carbopol 974P. As concentration of Carbopol 974P increases mucoadhesive force also increased. From the data, (table 7) it was also found that, by increasing formulation preparation temperature the mucoadhesive force increases. Earlier work with carbopol polymers has clearly indicated that it is the availability of carboxyl groups that determines bioadhesion. Carbopol has a very high percentage (58%- 68%) of carboxylic groups that gradually undergo hydrogen bonding with sugar residues in oligosaccharide chains in the mucus membrane, resulting in formation of a strengthened network between polymer and mucus membrane. Carbopol having high density of available hydrogen bonding groups would be able to interact more strongly with mucin glycoproteins. In addition, carbopol may also adopt more favorable macromolecular conformation with increased accessibility of its functional groups for hydrogen bonding. It is speculated that the higher mucoadhesive strength of the delivery system may lead to the prolonged retention and increased absorption across mucosal tissues.

In vitro diffusion study
In vitro diffusion studies of all formulations were done by using the Franz diffusion cell with 110 LA 395-1 MP (HiMedia laboratories Pvt. Ltd. Mumbai) as a dialysis membrane. For this study Phosphate buffer of pH 6.4 was used as diffusion media. Diffusion profiles of formulation series are elaborated in table 7.5 and figure 7.8. From the diffusion data it is shown that, when concentration of Carbopol 974P was used 0.5%,
then % diffusion for Donepezil hydrochloride was significantly lower as compared with that of other concentration of Carbopol 974P and when formulation preparation temperature was increased from 50C to 250C, then % drug diffusion decreased. This is because of the presence of Pluronic F-127 in the gel retards the drug release rate slightly owing to reduction in dimension of water channels resulting for enhanced micellar structure and its micellar structure becomes more rigid as a temperature of formulation preparation increased.

As concentration of Carbopol 974P increased in formulation more percent of drug diffused. It is because of presence of carbopol results in very rapid dissolution and release of highly soluble drug like Donepezil hydrochloride due to rapid swelling and dissolution of carbopol at pH 6.4. As seen from the results addition of 1.0% and 1.5% carbopol enhanced the diffusion of drug from gel significantly. This result could be attributed to increase in concentration of ionized carboxyl group to a level required to cause conformational changes in the polymer chain. Electrostatic repulsion of the ionized carboxyl group results in decoiling of the polymer chain, resulting in the relaxation of the polymer network. At this stage, drug is rapidly dissolved and released from the gels as a result of very high swelling or fast dissolution of the ionized carbopol.

![Figure 11: In-vitro release profile of all Batches](image_url)
Table 8: % Drug Diffusion Data of In Vitro Study of all batches.

<table>
<thead>
<tr>
<th>Time in Hours</th>
<th>% Drug Diffusion Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>F2</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>14.58±1.1</td>
</tr>
<tr>
<td>2</td>
<td>27.43±0.6</td>
</tr>
<tr>
<td>3</td>
<td>39.93±0.6</td>
</tr>
<tr>
<td>4</td>
<td>61.81±0.6</td>
</tr>
<tr>
<td>5</td>
<td>68.05±0.6</td>
</tr>
<tr>
<td>6</td>
<td>73.61±0.6</td>
</tr>
<tr>
<td>7</td>
<td>85.41±1.8</td>
</tr>
<tr>
<td>8</td>
<td>95.83±1.8</td>
</tr>
</tbody>
</table>
SUMMARY AND CONCLUSION
The present work was taken up to use the gel forming solution of an temperature sensitive polymer Pluronic F-127, together with the mucoadhesive polymer such as Carbopol 974P in order to develop a nasal in situ gel of Donepezil which can be expected to prove beneficial for overcoming the limitations of oral administration route like first pass metabolism of drug, side effects of drug after its oral administrations like fatigue, diarrhea, nausea, vomiting, etc. From this study, it is concluded that, among all formulation prepared F2 was the best optimized formulation. Which showed pH- 5.4, gelation temperature- 33.7°C, 99.15 % drug content, viscosity 19800 CP at 37°C, 48 sec. gel strength, 3857-29 dyne/cm² and after 8 hrs. it shows 99.31% drug diffusion. Hence, 18% Pluronic F-127 gel formulation with 1.0 % carbopol 974P which prepared at 50°C is a promising nasal drug delivery system for the anti-Alzheimer drug Donepezil, which would enhance nasal residence time owing to increased viscosity and mucoadhesive characteristics; furthermore, it also exhibited a permeation enhancing effect.

REFERENCES