ORIGINAL ARTICLE

# Fabrication of an Ion-sensitive *in situ* Gel loaded with Nanostructured Lipid Carrier for Nose to Brain Delivery of Donepezil

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## Abstract

**Aim:** The purpose of the present study was to formulate *in situ* gelling formulation combining nanostructured lipid carrier of donepezil and an ionic-triggered gellan gum matrix following intranasal administration for the treatment of Alzheimer's disease. **Materials and Methods:** Donepezil-loaded lipid carrier was prepared by a melt emulsification-probe sonication method, optimized, and characterized. Nanostructured lipid carrier was incorporated into gellan gum-based *in situ* gel and characterized for *in situ* gelling properties, rheological properties, and texture analysis. The developed formulation was evaluated *in vivo* through pharmacodynamic and pharmacokinetic study in rats. **Results and Discussion:** *In vivo* efficacy tested in scopolamine-induced amnesia model indicated a significant improvement in cognitive function in rat treated with *in situ* gel as compared to the marketed formulation. A pharmacokinetic study showed the higher drug distribution in brain and lower drug concentration in plasma from a developed formulation which indicates its higher safety and efficacy through a nasal route. **Conclusion:** Thus, nanostructured lipid carrier-based *in situ* gel administered through intranasal route can be clinically promising approach for enhancing efficacy through the brain and reducing toxicity to other organs.

**Key words:** Donepezil, gellan gum, *in situ* gel, nanostructured lipid carrier, nasal route

## INTRODUCTION

Alzheimer's disease is a neurodegenerative disorder, resulting in a memory loss, changes in personality, and incapability for self-care.[1,2] The existence of constricted connections in blood–brain barrier served as main barrier for drug delivery and attaining desired therapeutic concentration into the brain to treat Alzheimer's disease.[3]

Donepezil is available as twice a daily immediate release (5 or 10 mg) and once a daily the sustained release (23 mg) tablet in the market, intended for oral administration for the management of Alzheimer's disease.[4] Nevertheless, the oral administration of donepezil results in gastrointestinal side effects such as nausea, vomiting, and diarrhea.[5] Furthermore, it is rapidly absorbed and reaches to $C_{max}$ followed by a continuous decrease in the plasma concentration until the administration of the next dose. Thus, it does not maintain therapeutics concentration for a long duration. However, Alzheimer's patients suffer from dementia, and hence for next dose, they have to depend on caretaker or health-care professional. To overcome this, an alternative approach is essential which can maintained therapeutic concentration into the brain for long period of time with reduced dosing frequency. Considering the patients' need, intranasal administration of drug through lipid carrier can be an ideal approach for Alzheimer's disease.[6,7]

Incorporation of donepezil into nanocarrier, especially lipid carrier, can further improve the brain concentration due to lipidic nature of olfactory lobe. Hence, nanostructured lipid carrier is explored as drug delivery system owing to its potential to entrap the higher amount

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of drug as well as higher stability over solid lipid nanoparticles.[8-11]

The real challenge is administration and retention of the preparation for an extended period of time at nasal cavity. *In situ* gel is introduced as low viscosity solution into the nasal cavity, and the gelling agent forms a gel which prolongs contact time and releases the drug slowly and continuously.[12] In the current formulation, low acyl gellan gum was used as a gel-forming agent. It forms a gel on contact with cations such as sodium, potassium, and calcium of nasal fluid.[13] Hence, the rationale of this formulation is on instillation into the nasal cavity gellan gum-based solution which forms an *in situ* gel and will retain for long period of time.

The goal of the present research work was to develop nanostructured lipid carrier of donepezil, which would enhance drug residence time in the brain. To overcome quick removal of the donepezil from the nasal cavity and extend its nasal residence, the nanostructured lipid carrier of donepezil dispersed into in situ gel system containing gelling agent. Furthermore, the intranasal administration of developed *in situ* gel system was compared for pharmacodynamic and pharmacokinetic performance with the marketed formulation in a suitable animal model.

## MATERIALS AND METHODS

### Materials

Donepezil was kindly gifted by Ranbaxy Research Laboratory, Gurgaon, India. Glyceryl distearate was obtained as gift sample from Gattefosse India Ltd., Mumbai, India. Capmul MCM was supplied as gift sample from Abitec Corporation Ltd., Mumbai, India. Poloxamer 188 and Tween 80 were gifted by BASF Ltd., Mumbai, India. Gellan gum and xanthan gum were supplied as gift samples by C. P. Kelco, Mumbai, India. All reagents and reagents of analytical reagent grades were purchased from CDH, Mumbai, India. All the solvents of chromatographic grade were purchased from Merck, Mumbai, India.

### Methods

#### *Formulation of nanostructured lipid carrier*

Nanostructured lipid carrier was prepared using a method as mentioned in the literature.[14] An accurate amount of glyceryl distearate (lipid), Capmul MCM (oil), and AcrysolK150 (solubilizer) was melted at 5°C above the melting point of the solid lipid. Then, donepezil was added to melted blend and subjected to vortexing (Remi, Mumbai, India) for 2 min. Concurrently, a mixture of surface active agents (Tween 80 and Poloxamer 188) was added in distilled water and heated to the same temperature as that of lipid phase. Transparent primary emulsion was formulated by adding surfactant solution into the liquid phase and sonicated by probe sonicator (Sonics and Materials, CT, USA) for 2 min (3:2 s on:off pulse cycle) at an amplitude of 30%. The composition and evaluation of donepezil nanostructured lipid carriers (NLC) are shown in Tables 1 and 2, respectively.

#### *Characterization of nanostructured lipid carrier*

##### *Particle size, polydispersity index, and zeta potential*

The mean particle size, polydispersity index, and zeta potential determination were carried out using the principle of dynamic light scattering (DLS) by Zetasizer (Malvern Instruments, Malvern, UK). Samples ($n$ = 3) were diluted 10 times with distilled water, and parameters were determined using a 2 mL sample at 25 ± 2°C at scattering angle of 90°.[15]

##### *Drug loading and entrapment efficiency*

The drug loading and entrapment efficiency of nanostructured lipid carrier were measured by centrifugation method (Remi centrifuge, Mumbai, India). 2 mL of nanostructured lipid carrier dispersion was spinned at a speed of 20,000 rpm at 4 °C for 1 h and top portion was collected and suitably diluted with water, and free drug was determined using high-performance liquid chromatography. The percentage of drug loading and entrapment efficiency was determined using equation (1) and equation (2), respectively.[16]

$$\% \text{ Drug loading} = \frac{\text{Total amount of donepezil} - \text{Amount of donepezil in supernatant}}{\text{Total amount of lipid}} \times 100 \quad (1)$$

$$\% \text{ Entrapment efficiency} = \frac{\text{Total amount of donepezil} - \text{Amount of donepezil in supernatant}}{\text{Total amount of donepezil}} \times 100 \quad (2)$$

##### *Transmission electron microscopy (TEM)*

The surface morphology of nanostructured lipid carrier was studied using the TEM (Philips, Tokyo, Japan). The nanostructured lipid carrier dispersion (5–10 µl) diluted 10 times was placed on a carbon-coated copper grid which was then negatively stained by 1% (w/v) phosphotungstic acid solution and observed at 200 kV.[16]

#### Preparation of nanostructured lipid carrier loaded *in situ* gel

A clear solution of gellan gum was prepared in distilled water by heating at 90 °C with moderate stirring (Remi, Mumbai, India) and cooled. Simultaneously, xanthan gum was added to distilled water with stirring. Thereafter, NLCs were centrifuged and formed pellet was incorporated into the xanthan gum solution slowly with stirring. This

resulting solution was mixed with gellan gum solution and evaluated.[12]

### Characterization of nanostructured lipid carrier-loaded *in situ* gel

#### *Critical ionic concentration (CIC) for gellan gum phase transition*

The CIC was measured by mixing 1 mL of developed *in situ* gel with different quantities of simulated nasal fluid (composed of 1.29 mg/mL KCl, 7.45 mg/mL NaCl, and 0.32 mg/mL $CaCl_2.2H_2O$)[17] in test tubes. Then, test tubes were inverted and observed for gel formation. The minimum quantity of simulated nasal fluid required for gel formation was considered as the CIC ($n = 3$).[18]

#### *Gelling time*

The gelling time was determined by adding *in situ* gel (1 mL) ($n = 3$) into the specific quantity of simulated nasal fluid, and the period needed for the gel formation was recorded.[12]

#### *Expansion coefficient (S %)*

It was determined by mixing 1 mL of developed *in situ* gel with 0.25 mL simulated nasal fluid in a calibrated cylinder with the total initial volume of liquid as 1.25 mL ($V_1$). After addition of 2 mL of nasal fluid, the volume of gel was recorded as the total volume $V_T$. Thereafter, using volume of gel ($V_G = V_T$-2), expansion coefficient was calculated as follows ($n = 3$),[18]

$$\text{Expansion coefficient } (S\ \%) = \frac{VG - V1}{V1} * 100 \quad (3)$$

#### *Viscosity*

The viscosity of *in situ* gel before and after gelation was determined using Brookfield Viscometer (Brookfield Engineering Labs. Inc., MA, USA). The sample (25 mL) was taken in a beaker, and viscosity measurement was carried out at a speed of 10 rpm for 10 s at 25°C in the sol and gel state in triplicate.[19]

#### *In vitro mucoadhesion studies*

The mucoadhesive strength of the *in situ* gel was measured by texture analyzer (Brookfield Engineering Labs. Inc. MA, USA) with mucoadhesive holder. Sheep nasal mucosa (about 10 mm × 10 mm) was allowed to equilibrate for 10 min at 32±2°C in 20 mL of the simulated nasal fluid. 20 mg *in situ* gel was placed onto the mucoadhesive holder. The probe was lowered at a rate of 0.5 mm/s until it touched the membrane. A contact force of 1N was maintained for 30 s, and the probe was subsequently withdrawn at a rate of 0.5 mm/s to a distance of 20 mm. The maximum force required to separate the probe from the tissue (i.e., maximum detachment force in grams; $F_{max}$) could be recorded directly using Texture Pro CTV1.3 Build 14 Software.[20] The formula used to calculate mucoadhesive strength is as follows:

$$\text{Mucoadhesive strength } \left(\text{dyne} / \text{cm}^2\right) = \frac{\text{m.g}}{\text{A}} \quad (4)$$

Where m is the weight required for detachment (g), A is the area of tissue exposed ($cm^2$), and g is the acceleration due to gravity (980 $cm/s^2$)

### *Pharmacodynamic study*

#### *Selection and acclimatization of animals*

Throughout the experiment, animal handling was done as per the CPCSEA guidelines. The experimental procedure of the study was sanctioned by the Animal Ethical Committee of the Institute of Pharmacy (IP/PCEU/PHD/18/017). 4–6-week-old male Sprague-Dawley rats of 200–250 g weight were used for the study. The animals were kept at 20±2°C with 12 h light and dark cycle. All the animals were categorized into four groups each consists of six animals. Normal control as negative control group. 1 mg/kg scopolamine was administered by intraperitoneal route (i.p.) in rats of Group A. Group B rats were treated with *in situ* gel (1 mg/kg) by intranasal route (i. n.). In Group C, rats were treated with donepezil marketed formulation (1 mg/kg) by oral route.

#### *Experimental protocol*

The scopolamine administration leads to amnesia in rats. Morris water maze model is utilized to understand learning ability and memory of rat. The procedure, technique, and endpoint measurement were followed as described by Parle and Singh.[21] In this study, water maze (rat) consists of a circular tank (150 cm in diameter and 45 cm in height). A platform (12 cm in diameter and 28 cm high) invisible to the rat is set 2 cm under the water level inside the tank. The temperature of the tank was maintained at 20±2°C. The location of the platform remains constant throughout the study. The rat is taught to reach the center of the platform from any starting point (North, South, East, and West) of the tank.[3]

#### *Procedure*

Then, training was given 4 days consecutive to rats from different starting points. 30 min before trial on the 5$^{th}$ day, scopolamine (1 mg/kg i. p.) was administered to Groups A, B, and C except normal control group. 30 min before scopolamine injection, *in situ* gel was administered through the intranasal route using a polyurethane tube (24G × 19 mm) attached to the tip of micropipette and rats were kept in straight position so that maximum concentration of drug can reach to the brain.[22,23] Similarly, donepezil marketed formulations were crushed, and after suspending in saline water, it was administered in Group C animals by oral gavage 30 min before scopolamine injection. After 2 h of the treatment all Groups A, B and C were evaluated for; time taken (escape latency in second), distance travelled (path length in centimeter), time utilized in central platform, and number of crossings for the period of 90 s were recorded.[24]

### *Pharmacokinetic studies*

The various pharmacokinetic parameters were studied using male Sprague-Dawley rats. The rats were kept at 20±2°C for 12 h light and dark cycle. The animals were given food and water and were supervised for behavioral changes throughout the experiment. The rats were fasted overnight before starting the study.

All the animals were categorized into three groups each consists of 18 animals. In Group 1, rats of normal control group were included. In Group 2, rats were administered with *in situ* gel (1 mg/kg) by i.n. route. Group III consists of donepezil marketed formulation (1 mg/kg) by oral route.

Formulations were administered as per procedure mentioned in pharmacodynamic study. Three rats each were sacrificed at 0.5, 1, 2, 4, 6, and 8 h, and blood and other vital organs such as the brain, heart, liver, spleen, lung, and kidneys were collected for donepezil analysis.

Depending on the donepezil level in the brain and plasma level at different time intervals, different pharmacokinetic parameters were determined using Kinetica Software (non-compartment modeling) (Thermo Fisher Scientific, Waltham, USA, trial version 5.0) and statistical analysis was performed by GraphPad Prism Statistical Software (GraphPad Software Inc. La Jolla, CA, USA, version 5.03).[25] Pharmacokinetic parameters such as $C_{max}$, $T_{max}$, $AUC_{0-8}$, $AUC_{0-\infty}$, $AUMC_{0-8}$, MRT, and $K_{el}$ were determined for both the *in situ* gel and marketed formulation groups for the brain and plasma.

### Statistical analysis

Statistical data were represented as mean ± SD ($n$ = 3). All data was compared and analysed using a one-way analysis of variance (ANOVA) and Student's (paired) $t$-test followed by Tukey's *post hoc* test using GraphPad Prism Software. $P < 0.05$ was considered to be statistically significant.[25]

## RESULTS AND DISCUSSION

### Formulation of nanostructured lipid carrier

The nanostructured lipid carrier using glyceryl distearate (lipid) and Capmul MCM (oil) was formulated by melt emulsification-probe sonication method. The batches were prepared with alone Poloxamer 188 or Tween 80; however, these were not stable and showed higher particle size. Poloxamer 188 and Tween 80 both were considered as safe and biodegradable surfactants used to increase the stability of the formulation. In addition to that, Tween 80 has been used in tacrine, doxorubicin, dalargin, loperamide, and tubocurarine nanoparticles to improve brain delivery by increasing LDL-mediated endocytosis.[26] Nanostructured lipid carrier was prepared using varying lipid:oil ratios (1:1–1:5), sonication amplitude (10–50%), and sonication time (1–5 min) (data not shown). Batches prepared with the high amplitude of 50% for 2 min resulted in an aggregation of particles. It was observed that lipid:oil ratios of 1:1 and 1:2 with 30% amplitude for 2 min showed promising result, i.e., particle size <200 nm.[19] Formulation with transparent appearance and bluish ting is considered as stable, and hence, Acrysol K 150 was added in all the batches as solubilizer and stabilizer. Various batches were prepared as shown in Table 1 to understand the effect of each excipient.

### Characterization of nanostructured lipid carrier

#### *Particle size, polydispersity index, and zeta potential*

The stability of the nanostructured lipid carrier was determined by measuring the physical properties such as mean particle size and zeta potential. The amount of total lipid and surfactant affects the particle size. All the batches showed particles size <200 nm; however, with the increase in a surfactant concentration, it reduces to as low as 112 nm in batch D6. As the concentration of liquid lipid increased, the decrease in particle size was observed (Batch D4). This might be due to expelling out of excess lipid during particle formation.[27] Polydispersity index <0.300 indicates narrow and uniform particle size distribution.[28] The uniformity in particle size was also increased with the increase in the concentration of surfactant as reflected in batches D5 and D6 in Table 2.

Further, zeta potential is an important parameter which is directly related to the physical stability of colloidal systems.[29] In theory, higher positive or negative values of zeta potential lead to stabilization of such systems. The electrostatic repulsion between particles with the same electrical charge results in less aggregation of particles with pronounced zeta potential (>|20|).[30] In this study, optimized batch (D6) showed zeta potential of −35 mV, which provides sufficient electrostatic repulsion and thus stabilizes the system. This might be due to ionization or hydrolysis of a fatty ester group of glyceryl distearate.[31]

**Table 1:** Composition of donepezil nanostructured lipid carrier batches

| Name of ingredient | Batch No | | | | | |
|---|---|---|---|---|---|---|
| | D1 | D2 | D3 | D4 | D5 | D6 |
| Donepezil | 5 | 5 | 5 | 5 | 5 | 10 |
| Glyceryl distearate | 50 | 50 | 100 | 100 | 100 | 100 |
| Capmul MCM | 50 | 50 | 50 | 100 | 100 | 100 |
| Acrysol K150 | NA | 100 | 100 | 100 | 100 | 100 |
| Poloxamer188 | 100 | 100 | 100 | 100 | 150 | 150 |
| Tween80 | 40 | 40 | 40 | 40 | 40 | 40 |

The quantities are expressed in mg

### *Drug loading and entrapment efficiency*

Drug loading and entrapment efficiency are mainly depending on the nature of the drug and the lipid in which drug is encapsulated. If lipid alone is used, the amount of drug can be loaded into it is limited, and hence, a combination of lipid and oil is used in nanostructured lipid carrier which makes it superior over solid lipid nanoparticles.[32] The incorporation of Capmul MCM (oil) to the glyceryl distearate (lipid) resulted in increased entrapment efficiency. The increase in entrapment efficiency might be due to the reduction in crystallinity of particles which is responsible for improving the stability of the formulation.[33] The increase in the lipid content resulted in an increase in the entrapment efficiency by providing additional space to incorporate the drug and reducing the escaping of the drug into the surfactant phase during preparation.[34]

The higher entrapment efficiency was observed for nanostructured lipid carrier containing both surfactants (Poloxamer 188 and Tween 80) compared to Poloxamer 188 alone. This might be due to the better stabilization of primary emulsion by the surfactant mixture (Poloxamer 188 and Tween 80). The risk of escaping of donepezil from the lipid core to the surfactant part was observed due to the insufficient emulsification when Tween 80 was used alone. Hence, combinations of surfactants were used for further study. With an increase in the proportion of surfactants, entrapment efficiency was also increase as shown in Table 2 (Batches D5 and D6).

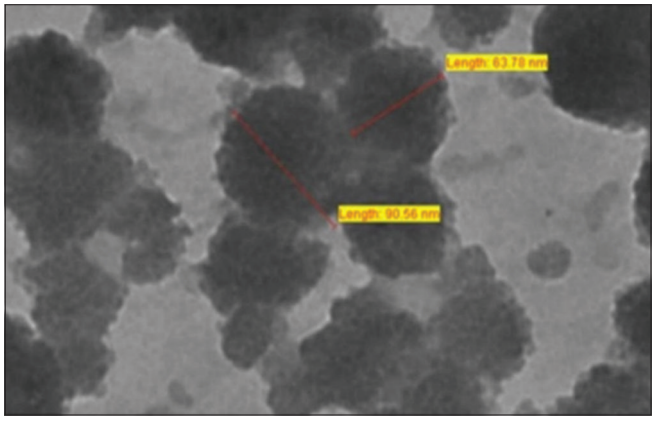


**Figure 1:** Transmission electron microscopy image of the optimized batch (D6)

### *TEM*

TEM was used to measure particle size and morphology accurately. As shown in Figure 1, NLCs were spherical in shape. The particle size measured by TEM analysis was comparable with DLS data, and only slightly smaller size particles were observed in TEM. It also showed spherical and uniform size particles.[16]

### Preparationof nanostructured lipid carrier-loaded *in situ* gel

The clear, transparent gel with good gel properties was prepared using gellan gum as a gelling agent and xanthan gum as a gum base. The different concentrations of gellan gum 0.25%, 0.5%, and 1% w/v were used to formulate batches. A 0.25% w/v concentration of gellan gum was not enough to form a gel and 1% w/v concentration of gellan gum resulted in gel with very high gel strength and also stiff gel. The batches prepared with 0.5% w/v concentration of gellan gum showed clear, translucent gel with appropriate mucoadhesive strength and best viscosity.

### Characterization of nanostructured lipid carrier-loaded *in situ* gel

The CIC of gellan gum is an important component for the characterization of ion activated *in situ* gel, as it is an ion-sensitive polymer. A sudden phase transfer from solution phase to gel phase will occur by means of ionic mechanism, when cation present in the gel dominates the CIC.[18] The CIC of the gel was determined using artificial simulated nasal fluid. The nasal cavity contains a very less quantity of simulated nasal fluid; hence, it is very important to determine the quantity of simulated nasal fluid needed for the formation of a gel. The minimum quantity of simulated nasal fluid required for gel formation was considered as the CIC, and the result of CIC is shown in Table 3.

The gelling time indicates the period entailed for the formation of the gel. The minimum time taken by the sol to convert into gel was considered as the best gel. It is nothing but the time taken by gellan gum to form a complex with components of the simulated nasal fluid, especially sodium,

**Table 2:** Characterization of nanostructured lipid carrier batches

| Batch No | Particle size (nm) | Polydispersity index | Zeta potential (mV) | Percentage drug loading | Percentage encapsulation efficiency |
|---|---|---|---|---|---|
| D1 | 190±9.88 | 0.402±0.005 | −12±6.03 | 3.52±1.49 | 35.24±5.53 |
| D2 | 160±8.12 | 0.312±0.006 | −8±4.93 | 4.52±2.04 | 45.18±8.26 |
| D3 | 172±9.00 | 0.328±0.008 | −4±3.21 | 2.96±0.93 | 59.45±7.55 |
| D4 | 120±8.40 | 0.213±0.004 | −18±7.21 | 3.61±1.37 | 72.16±7.31 |
| D5 | 103±6.51 | 0.138±0.004 | −27±8.62 | 4.34±1.88 | 86.81±6.39 |
| D6 | 112±9.39 | 0.114±0.007 | −35±7.55 | 7.93±2.13 | 79.25±3.77 |

Mean ± SD (*n*=3)

**Table 3.Characterization of *in situ* gel batches**

| Batch No | Critical ionic concentration (%) | Gelling time (s) | Expansion coefficient (%) | Viscosity (cp) | | Mucoadhesive strength (dyne/cm²) |
|---|---|---|---|---|---|---|
| | | | | Before gelation | After gelation | |
| D1 | 0.53±0.05 | 50±6.66 | 1.45±0.06 | 6.32±2.50 | 52.30±7.27 | 1954±67.55 |
| D2 | 0.41±0.03 | 25±5.03 | 1.23±0.05 | 5.54±3.41 | 47.57±8.07 | 2265±57.81 |
| D3 | 0.27±0.04 | 32±6.51 | 1.63±0.04 | 3.15±1.62 | 34.23±7.24 | 2154±94.74 |
| D4 | 0.35±0.06 | 43±7.77 | 1.34±0.06 | 4.29±2.80 | 40.60±5.41 | 1645±78.54 |
| D5 | 0.18±0.06 | 25±6.11 | 1.76±0.05 | 11.52±6.36 | 44.13±5.82 | 2652±93.51 |
| D6 | 0.10±0.04 | 19±3.51 | 1.62±0.04 | 2.32±1.22 | 48.27±6.40 | 3189±84.26 |

Mean±SD (*n*=3)

calcium, and potassium ions.[35] All the batches showed quick gel formation (gelling time less than a minute).

When the solution converted into a gel due to the interaction of gellan gum with ions of simulated nasal fluid, increase in its volume was observed. The solution-gel phase transition resulted in the formation of is more quantity of the gel than the solution. Hence, it is important to calculate the expansion coefficient as mentioned.[18] Swelling of gel results into expansion and discomfort feeling in small nasal cavity. The highest limit of expansion coefficient was only about 3%. This slight expansion may not cause uneasiness to the patient after administration of gel through the nasal route.[12] The expansion coefficient of all batches was <3%.

Intranasal absorption depends on the retention time of formulation at the olfactory regions located in the upper nasal surface. From absorption point of view, the rheological properties of *in situ* gel make it formulation of choice over conventional nasal formulation.[36] The high viscosity could extend the residence time on mucous membrane, preferring drug absorption.[37] The sol should have an optimum viscosity which facilitates administration. Then, sol undergoes a rapid sol-gel transition, resulting in the formation of the gel at a target site due to its ionic interaction.[38] In addition, the *in situ* gel had enough strength to release drug slowly over 8 h without degrading. The viscosity of sol was between 2 and 11 cp which was increased after addition to simulated nasal fluid in the range of 34–52 cp [Table 3].

The mucoadhesive strength of the formed gel was determined using a texture analyzer. The higher mucoadhesive strength was observed due to the binding of nanostructured lipid carrier components to the negatively charged groups (such as carboxyl and sulfate) on the mucin and cell surface according to the electronic theory of mucoadhesion.[16] The mucoadhesive strength of the optimized *in situ* gel batch was 3189 dyne/cm$^2$. The composition and evaluation parameters in sol and gel form for various prepared nanostructured lipid carrier loaded *in situ* gels are mentioned in Table 3. The drug content of all the batches were ranges from 76% to 98% w/w.

## Pharmacodynamic study

Morris water maze test is a sensitive method for showing the destruction of learning and memory. All the rats significantly shortened escape latency during the acquisition training, and there was no significant variation in the escape latencies between the groups tested on the same day.

On the 5$^{th}$ day, before actual test, scopolamine injection was given to Group A, B, and C rats to produce memory loss. Before 30 min of scopolamine administration, optimized *in situ* gel and marketed formulation were administered to Group B and C rats, respectively.

The escape latency time was used as a parameter for evaluating performance in tested rats in the Maze test. For every rat, the escape latency for four trials was averaged, and the results are shown in Figure 2a. The normal control group rats showed smaller escape latency time (46 ± 5.10 s) compared to the group treated with scopolamine (Group A) (79 ± 7.45 s). This clearly indicates the amnesia generated by scopolamine. The Groups B and C were treated with optimized *in situ* gel and marketed formulation, respectively, inverse the memory loss induced by scopolamine with shorter escape latency time of 52 ± 5.35 s and 64 ± 6.87 s, respectively. The lower value of latency time of developed formulation over marketed indicates the effectiveness of it. However, the multiple-dose study could better explain the purpose.

In the probe trial test, comparison between groups was done by calculating the distance traveled for each rat, and the results are shown in Figure 2b. Groups B and C showed a significant decrease in the distance travelled in comparison with the rats of Group A with amnesia.

The time spent in target quadrant was determined for all three groups, and Group B and Group C showed more time spent in comparison with the rats of Group A with amnesia as shown in Figure 2c.

The significant difference in annulus crossings was observed with Groups B and C in comparison with the rats of Group A with amnesia as shown in Figure 2d.

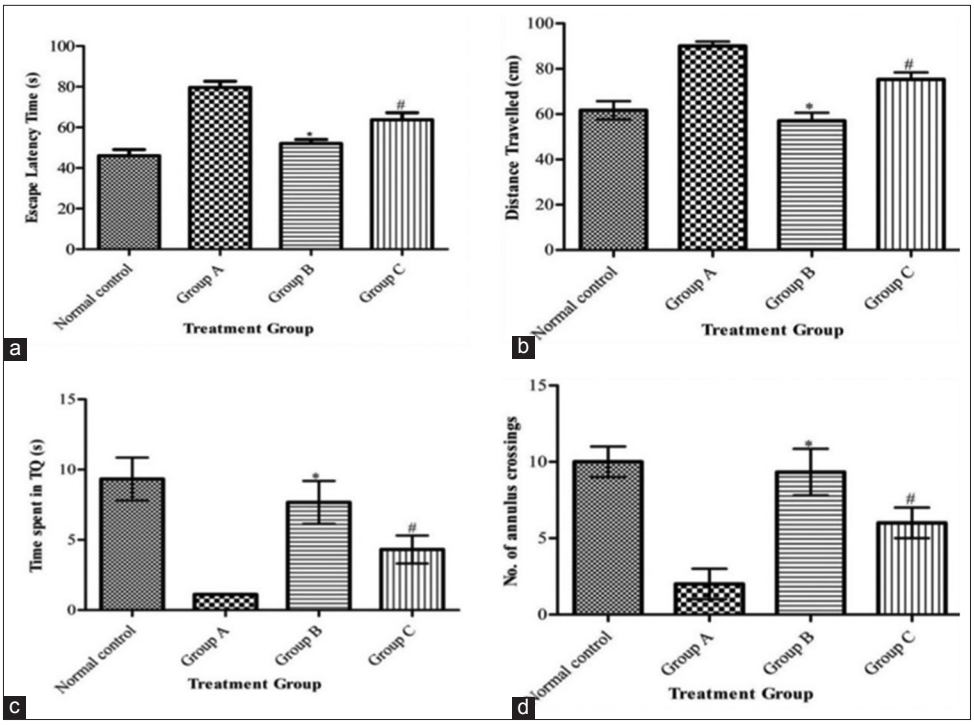


**Figure 2:** Pharmacodynamic study in rats using Morris water maze test. Values are mean ± SD of ($n$ = 6). Significant difference by one-way ANOVA followed by Tukey's multiple comparison test; *$P$ < 0.05 versus scopolamine-induced amnesia; #$P$ < 0.05 versus optimized *in situ* gel. Group A - scopolamine-induced amnesia, Group B - optimized *in situ* gel (R6), Group C - donepezil suspension (a) Escape latency time, (b) distance travelled; (c) total time spent in target quadrant, (d) number of annulus crossings

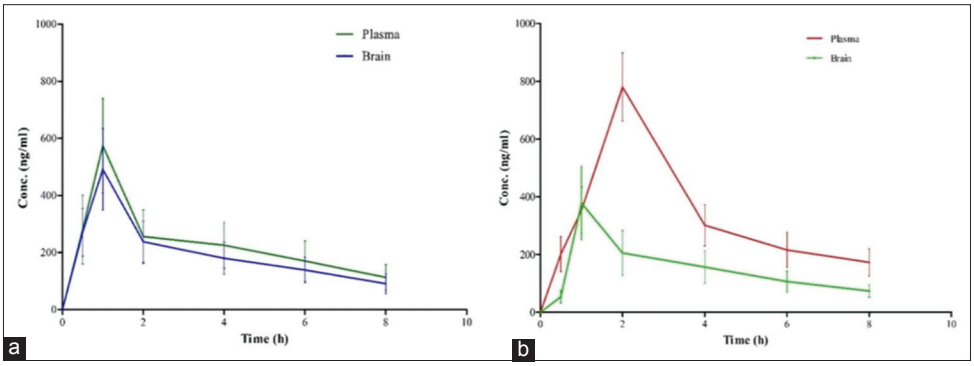


**Figure 3:** Donepezil concentration versus time profile in plasma and brain. (a) Donepezil nanostructured lipid carriers-based gel (D6) through nasal administration, (b) donepezil marketed formulation via oral administration

## Pharmacokinetic study of donepezil in brain and plasma

After administration of developed optimized *in situ* gel (Batch D6) and marketed formulation, drug concentrations were measured in the brain and plasma at regular intervals and area under the curve was drawn as shown in Figure 3a and b, respectively.

Here, $t_{max}$ is around 1 h, this delay in $t_{max}$ may be due to gelling matrix of gellan gum may be providing initial resistance for the penetration of nanostructured lipid carrier. However, as residence time increases, penetration was also increases significantly.

The maximum concentration ($C_{max}$) was found to be higher after intranasal administration of *in situ* gel compared to *in situ* marketed formulation; however, reverse results were observed in plasma [Table 4]. Further AUC also indicated the higher concentration of donepezil in the brain after nasal administration. From developed *in situ* gel, approximately equal amount of drug went into plasma and brain, while plasma concentrations were doubled to that of the brain concentration in case of marketed formulation. Thus, we can conclude that partially donepezil might have been absorbed through olfactory lobe. Here, nanostructured lipid carrier of donepezil acts as a carrier system due to its lipidic nature as well as the lipidic nature of nasal mucosa. In addition, *in situ* gelling system of gellan gum provides long residence time. Hence, the developed formulation is worked synergistically, which may be responsible for higher concentration in the brain after nasal administration. The mean residence time was

**Table 4:** Pharmacokinetic parameters of donepezil concentration in the plasma and brain after the administration of optimized *in situ* gel and marketed formulation at a dose of 1 mg/kg using intranasal and oral route respectively

| Pharmacokinetic parameters | Plasma | | Brain | |
|---|---|---|---|---|
| | *In situ* gel | Marketed formulation | *In situ* gel | Marketed formulation |
| $t_{max}$ (h) | 1 | 2 | 1 | 1 |
| $C_{max}$ (ng/ml) (ng/g) | 573.99±25.74 | 779.81±32.55 | 491.00±44.44 | 378.12±27.17 |
| $AUC_{0\rightarrow 8}$ (ng h/mL) (ng h/g) | 1858.93±368.84 | 2742.99±591.92 | 1538.21±266.84 | 1218.23±199.53 |
| $AUC_{0\rightarrow \infty}$ (ng h/mL) (ng h/g) | 2748.71±528.43 | 3351.83±441.81 | 2143.54±289.50 | 1652.33±349.35 |
| $AUMC_{0\rightarrow 8}$ (ng $h^2$/mL) (ng $h^2$ g) | 19665.70±4529.65 | 16419.00±3152.00 | 13654.60±2428.75 | 10017.00±2472.68 |
| MRT (h) | 7.15±2.15 | 4.89±2.37 | 6.37±2.49 | 6.06±2.09 |
| $K_{el}$ ($h^{-1}$) | 0.136±0.036 | 0.242±0.026 | 0.157±0.034 | 0.172±0.018 |

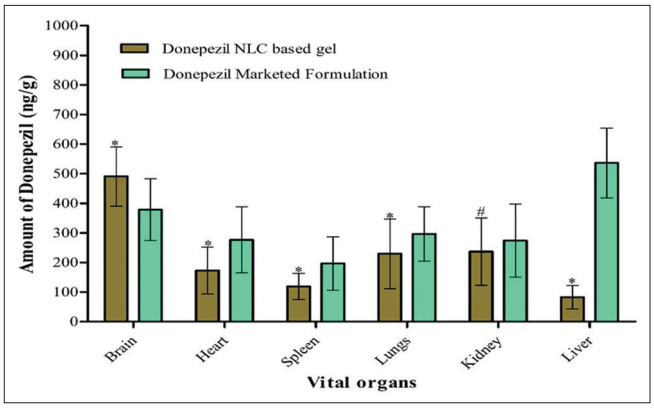


**Figure 4:** Quantity of donepezil per gram of the brain, heart, spleen, lungs, kidney, and liver after intranasal administration (1 mg/kg) of donepezil nanostructured lipid carriers based *in situ* gel (D6) and after oral administration (1 mg/kg) of donepezil marketed formulation; mean ± SD ($n$ = 6)

higher for developed formulation than marketed formulation in plasma as well as the brain. This may be due to lipid nature of formulation and *in situ* gel which increases drug retentions as well as slower drug release from nanostructure.

Biodistribution in different vital organs was also studied for developed *in situ* gel and marketed formulation after 60 min [Figure 4]. After nasal administration, the drug may enter into GI tract as well it may absorb through olfactory lobe into the brain.[39] The two-fold higher donepezil concentration in the brain after *in situ* gel nasal administration as compared to oral administration of marketed formulation further supports olfactory absorption of the drug. However, the liver showed significantly higher drug concentration after oral administration which may be due to first-pass metabolism and higher reticuloendothelial uptake of drug.[40] Tween 80 is also reported to retard drug uptake by organs, and its presence in *in situ* gel might also be responsible for lower drug concentrations in such organs.[41,42] The further significantly higher drug was also distributed into the heart, spleen, and lungs after oral administration, while the insignificant difference was observed in drug concentration in the kidney. Thus, nasal route can be considered as an alternative to oral route for the treatment of central nervous system diseases.

Significant difference by one-way ANOVA followed by Tukey's multiple comparison test; *$P$ < 0.001 versus donepezil marketed formulation (significant difference); #$P$ < 0.001 versus donepezil marketed formulation (no significant difference).

## CONCLUSION

The nanostructured lipid carrier-loaded *in situ* gel of donepezil has been prepared successfully by dissolving donepezil-loaded nanostructured lipid carrier into gellan gum 0.5% (w/v) and xanthan gum (0.15% w/v) solution. By studying the process and product parameters at the appropriate level, it was possible to prepare nanostructured lipid carrier-loaded *in situ* gel with desired properties, such as CIC, gelling time, expansion coefficient, viscosity, and mucoadhesion strength. The developed formulation served many purposes, increased absorption due to the presence of lipid in the formulation, and higher retention time at olfactory lobe by incorporating nanostructured lipid particles into *in situ* gel; Administration via nasal route bypass the first pass metabolism. Thus, developed nanostructured lipid carrier-loaded *in situ* gel can be considered as a suitable nasal delivery system for the administration of donepezil in the treatment of Alzheimer's disease.

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