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Research Article

## Development and evaluation of clozapine intranasal mucoadhesive in situ gels for brain targeting

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### ABSTRACT

Blood brain barrier, poor solubility and low bioavailability (<27%) due to hepatic metabolism are major constraints of clozapine (CZP) oral tablets for their clinical application in the treatment of psychosis. Therefore, the study aims to develop pluronic based thermosensitive mucoadhesive in situ gel of clozapine for brain targeting through intranasal olfactory pathway. The objective of the present study was to develop an aqueous and oil based thermosensitive mucoadhesive *in situ* gel of clozapine for intranasal delivery and to evaluate the gels for in vitro characterization and ex vivo permeation in comparison to drug solution. The aqueous and oil based *in situ* gel systems were developed by cold method using water and oleic acid as solvents respectively. Combination of Pluronic F-127 and F-68 (20:2) were used as thermosensitive gelling agents. Labrasol and Transcutol P at 1:1 ratio were employed as co solvents for the solubilisation of drug. The prepared *in situ* gels were evaluated for clarity, gelation temperature (*Tsol-gel*), gelation time (GT), gel strength (GS), pH, viscosity, mucoadhesive strength and ex vivo drug permeation studies. The effect of mucoadhesive agents like Chitosan, Sodium-β- glycerophosphate and Polyox WSR303 on gelation temperature (*Tsol-gel*) and drug permeation was also evaluated. The optimized aqueous *in situ* gel with 0.5% chitosan (Fa15) showed viscosity  $554.66 \pm 8.73$  cP at 31°C; mucoadhesive strength  $5114.91 \pm 107.37$  dynes/cm<sup>2</sup>, gelation temperature (*Tsol-gel*)  $29.6 \pm 1.7$  °C and gelation time (GT)  $69 \pm 5$  sec. The flux was found to be  $243.46 \mu\text{g}/\text{cm}^2/\text{hr}$  which was significantly high ( $p < 0.0001$ ) compared to drug solution and the enhancement ratio (ER) was found to be 2.28 folds to the drug solution whereas the oil gel showed flux of  $190.34 \mu\text{g}/\text{cm}^2/\text{hr}$  and enhancement ratio was found to be 1.64 folds to the drug solution. The results indicated that the hydrogels are potential carriers than oil gels for delivery of clozapine via intranasal route.

**Keywords:** Clozapine, *in situ* gels, nasal drug delivery, olfactory pathway, brain targeting, psychosis

**Article Info:** Received 19 Feb 2019; Review Completed 26 March 2019; Accepted 28 March 2019; Available online 15 April 2019

#### Cite this article as:



Ravikrishna V, Krishnaveni J, Development and evaluation of clozapine intranasal mucoadhesive in situ gels for brain targeting, Journal of Drug Delivery and Therapeutics. 2019; 9(2-s):198-207  
<http://dx.doi.org/10.22270/jddt.v9i2-s.2491>

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### 1. INTRODUCTION

The mental and behavioural disorders are chronic, severe and disabling brain disorders affecting people throughout life. In India, it is estimated that 6-7% population suffer from common mental disorders and 1-2% severe mental disorders. The economic burden of schizophrenia is particularly great; approximately, the treatment consumes a total of \$ 63 billion per year <sup>1,2</sup>.

Clozapine (CZP) is a prototype atypical antipsychotic drug used to treat patients with schizophrenia who are unresponsive or intolerant to typical antipsychotics. Clozapine acts through a combination of antagonistic effects at D2 receptors in the mesolimbic pathway and 5-HT<sub>2A</sub> receptors in the frontal cortex. D2 antagonism relieves positive symptoms while 5-HT<sub>2A</sub> antagonism alleviates negative symptoms. Clozapine is also more effective in resistant schizophrenia and reduces suicidal behavior in patients which is common with the case of older antipsychotics <sup>30,31</sup>.

The clozapine absorbed from oral tablets reach brain to small extent due to blood brain barrier and degradation in GI harsh environment and extensive hepatic first pass metabolism and hence poor bioavailability (<27) due to extensive first pass effect by CYP1A2 and CYP3A4. Many patients (69-82%) discontinue medication due to its intolerable side effects. Hence, it is worth exploring alternative routes of administration to avoid its first-pass metabolism as well as to enhance the bioavailability and brain targeting efficiency of CZP <sup>3</sup>.

In recent times, interest in intranasal route to target drugs to brain circumventing blood-brain barrier has gained importance <sup>4</sup>. This method works because of the unique connection between the nose and the brain through olfactory nerve pathway <sup>5</sup>. Intranasal drug administration has been considered to treat neuropsychiatric disorder due to the advantages brought by the highly vascularised nasal epithelium, which gives extraordinary permeability of drugs and therefore results in rapid drug absorption <sup>6</sup>. This approach can also avoid the first pass metabolism and provides a practical, non-invasive, rapid and efficient method

to deliver drug molecules to the brain <sup>5</sup>. Many of the researchers have proved and reported that various nasal formulations including microemulsions, nanoparticulates, microspheres and thermoreversible in situ gels can be employed to transport the drug molecules effectively to central nervous system (CNS) via olfactory and trigeminal neural pathways. However, the major problems that persist with nasal solutions are cleared off rapidly from nasal cavity due to mucociliary clearance. Therefore, designing an intranasal dosage form with mucoadhesive character is particularly helpful as it provides intimate contact between dosage form and nasal epithelium and increased residence time thereby maximizing the chances of absorption for a drug molecule <sup>4, 7</sup>. The in situ gelling polymers are suitable approaches to enhance the nasal bioavailability aim by prolonging the contact time with the nasal surface <sup>8</sup>.

An *in situ* gel system ('*In-situ*' is a Latin word which means '*in position*') is a drug delivery system that exhibits *sol- to -gel* phase transition due to change in specific physicochemical parameters such as *ionic strength, temperature or pH*. The principal advantage of in situ gels is that they can be easily instilled in liquid form and are capable of prolonging the residence time of the formulation on the surface of the nasal cavity due to gelling nature at nasal temperature <sup>7, 8</sup>.

The most commonly used thermoreversible in situ gels are those prepared from diblock polymers poly (ethylene oxide)-b-poly (propylene oxide)-b-poly (ethylene oxide) popularly known as: Pluronics, Tetronics, Poloxamers etc. Pluronic F 127 is widely used as a thermosensitive gelling agent since it is non-toxic, non-irritating, poses good release characteristics, has poor inherent mucoadhesive properties. Therefore, mucoadhesive polymer such as chitosan was added along with poloxamers to make effective intranasal in situ gel formulation <sup>4, 9, 10</sup>.

Therefore, in the present research work, intranasal thermoreversible mucoadhesive in situ gels of clozapine were developed using pluronic. The main objective behind this study is to increase the residence time of drug in nasal cavity by minimizing the anterior leakage of formulation and post-nasal drip, thereby maximizing the absorption of drug and its bioavailability to brain via olfactory pathway.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Clozapine was a kind gift from Aurobindo Pharmaceuticals Ltd (Hyderabad, India); Pluronics (PF-127, PF-68) were purchased from BASF Chemical Ltd (Chennai, India); Labrasol and Transcutol-P were gifted from Gattefosse Pvt. Ltd. (Mumbai, India); Chitosan (CH; low molecular weight) was purchased from Sigma-Aldrich (Bangalore, India); Oleic acid was purchased from SD fine chemicals Ltd (Mumbai, India); Sodium-β-glycerophosphate was purchased from Sisco research lab Pvt Ltd, (Tabja, India); Polyox WSR-303 was purchased from Dow chemical Pvt Ltd (Mumbai, India)

All other reagents were of analytical grade. Water purification system (Millipore, MA, USA) was used to obtain high quality water.

### 2.2. Preformulation studies

#### 2.2.1. Construction of standard graph of Clozapine (CZP)

The standard graph of clozapine was constructed in different solvents such as methanol, water, phosphate buffer saline pH 6.4, phosphate buffers of pH 5.5, 6.8 and 7.4 by using UV-visible spectrophotometer (Labindia, 3000+).

50 mg of the Clozapine was weighed accurately and transferred into the 50 mL volumetric flask. The drug was dissolved in sufficient quantity of solvent and volume was made up to the mark to get a 1 mg/mL (Stock-A). Stock-A was further diluted to obtain desired concentrations. The absorbance of samples was measured at 216 nm against blank. A standard graph was plotted by taking concentration (μg) on X-axis and absorbance (nm) on Y-axis <sup>11</sup>.

#### 2.2.2. Differential scanning calorimetry

Differential Scanning Colorimetry (DSC) of pure drug (Clozapine) was determined by using DSC instrument (Shimadzu, DSC W70). About 10 mg of CZP was weighed and sealed in standard DSC aluminum pans, crimped it and then scanned over a temperature range from 50 °C to 200 °C at a heating rate 10 °C/min. The empty aluminium pan was used as reference cell <sup>12</sup>.

#### 2.2.3. Solubility studies

An excess amount of drug was taken into vials containing 5mL of solvents. Vials were closed and constantly agitated at 37± 2°C for 48 hrs by using thermo regulatory water bath shaker (Remi Instruments, Mumbai). The sample was filtered through 0.2μm syringe filter and the amount of drug solubilized was estimated by UV method described earlier <sup>13</sup>.

### 2.3. Preparation and optimization of nasal *in situ* Gels

#### 2.3.1. Preparation of thermosensitive gel

The thermosensitive gel was prepared using cold method <sup>14, 15</sup>. The required amount of pluronic (PF-127) was slowly added into the required amount of cold purified water with continuous stirring for 15 min, and then the dispersion was kept overnight at 2-8°C until a transparent hydrogel was obtained. Then, the required amount of drug and mucoadhesive agent dissolved in co solvent mixture was added to preformed gel with continuous stirring. The final weight of preparation was adjusted with water in case of aqueous gels and with oleic acid in case of oily gels. The dispersion was stored in refrigerator overnight to obtain a clear and transparent gel. The composition of CZP *in situ* formulations was given in Table 1a (aqueous gels) & 1b (oil gels)

Table 1a: Composition of clozapine nasal mucoadhesive *in situ* gels (aqueous based)

| Ingredients<br>(%w/w) | Formulations |     |     |     |     |     |     |     |     |     |      |      |      |      |      |      |
|-----------------------|--------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|------|------|------|------|------|
|                       | Fa0          | Fa1 | Fa2 | Fa3 | Fa4 | Fa5 | Fa6 | Fa7 | Fa8 | Fa9 | Fa10 | Fa11 | Fa12 | Fa13 | Fa14 | Fa15 |
| CZP                   | 5            | 5   | 5   | 5   | 5   | 5   | 5   | 5   | 5   | 5   | 5    | 5    | 5    | 5    | 5    | 5    |
| PF-127                | -            | 20  | 20  | 20  | 20  | 20  | 20  | 20  | 20  | 20  | 20   | 20   | 20   | 20   | 20   | 20   |
| PF-68                 | -            | 2   | 2   | 2   | 2   | 2   | 2   | 2   | 2   | 2   | 2    | 2    | 2    | 2    | 2    | 2    |
| PWSR303               | -            | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 | -   | -   | -   | -   | -    | -    | -    | -    | -    | -    |
| Sod.β-GP              | -            | -   | -   | -   | -   | -   | 0.1 | 0.2 | 0.3 | 0.4 | 0.5  | -    | -    | -    | -    | -    |
| Chitosan              | -            | -   | -   | -   | -   | -   | -   | -   | -   | -   | -    | 0.1  | 0.2  | 0.3  | 0.4  | 0.5  |
| L:Tp (1:1)            | 25           | 25  | 25  | 25  | 25  | 25  | 25  | 25  | 25  | 25  | 25   | 25   | 25   | 25   | 25   | 25   |
| Water                 | q.s          | q.s | q.s | q.s | q.s | q.s | q.s | q.s | q.s | q.s | q.s  | q.s  | q.s  | q.s  | q.s  | q.s  |

Table 1b: Composition of clozapine nasal mucoadhesive *in situ* gels (oil based)

| Ingredients<br>(%w/w) | Formulations |     |     |     |     |     |     |     |     |     |      |      |      |      |      |      |
|-----------------------|--------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|------|------|------|------|------|
|                       | Fo0          | Fo1 | Fo2 | Fo3 | Fo4 | Fo5 | Fo6 | Fo7 | Fo8 | Fo9 | Fo10 | Fo11 | Fo12 | Fo13 | Fo14 | Fo15 |
| CZP                   | 5            | 5   | 5   | 5   | 5   | 5   | 5   | 5   | 5   | 5   | 5    | 5    | 5    | 5    | 5    | 5    |
| PF-127                | -            | 20  | 20  | 20  | 20  | 20  | 20  | 20  | 20  | 20  | 20   | 20   | 20   | 20   | 20   | 20   |
| PF-68                 | -            | 2   | 2   | 2   | 2   | 2   | 2   | 2   | 2   | 2   | 2    | 2    | 2    | 2    | 2    | 2    |
| PWSR303               | -            | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 | -   | -   | -   | -   | -    | -    | -    | -    | -    | -    |
| Sod.β-GP              | -            | -   | -   | -   | -   | -   | 0.1 | 0.2 | 0.3 | 0.4 | 0.5  | -    | -    | -    | -    | -    |
| Chitosan              | -            | -   | -   | -   | -   | -   | -   | -   | -   | -   | -    | 0.1  | 0.2  | 0.3  | 0.4  | 0.5  |
| L:Tp (1:1)            | 25           | 25  | 25  | 25  | 25  | 25  | 25  | 25  | 25  | 25  | 25   | 25   | 25   | 25   | 25   | 25   |
| Oleic Acid            | q.s          | q.s | q.s | q.s | q.s | q.s | q.s | q.s | q.s | q.s | q.s  | q.s  | q.s  | q.s  | q.s  | q.s  |

CZP-Clozapine; PF-Pluronic F; PWSR-Polyox WSR; Sod.β-GP-Sodium β glycerophosphate L: Tp-Labrasol: Transcutol P; Fa- aqueous based gel formulations; Fo-oil based gel formulations

### 2.3.2. Screening and optimization of PF-127& PF-68 Concentrations for gelation

The preliminary screening was carried out using different proportions of Pluronic-F127 (10-22% w/w) and Pluronic F-68(2-8%w/w) as shown in Table 2. The gels were prepared as described in section.2.3.1. The gelation temperature was determined at each combination as per the method described in 2.4.1. The proportion of PF-127/PF-68 which showed gelation temperature ( $T_{sol-gel}$ ) of 31-32°C was selected for further development of clozapine formulation <sup>16</sup>.

### 2.3.3. Preparation of Clozapine Solution (CZP-Plain)

The Clozapine solution was prepared by dissolving clozapine (5%w/w) in weighed amount of Labrasol and Transcutol-P (1:1)(25%w/w) and made the final weight with water for aqueous solution and with oleic acid for oily solution. The pH of the solution was adjusted to 5.5±0.2 using 0.1M HCl <sup>17</sup>.

## 2.4. Physicochemical characterization of prepared in situ gels

### 2.4.1. Determination of Gelation temperature ( $T_{sol-gel}$ ) and Gelation time (GT)

The gelation temperature of formulation was determined by tube inversion method <sup>18</sup>. The prepared formulation (~1g) was transferred to a test tube and the gelling temperature was measured by immersing it in a thermo regulatory water bath. The temperature was increased from 25°C to 37°C gradually with an increment of 1°C until gelation. The temperature was equilibrated for 5 min at each point and the test tube was removed and inverted immediately to observe the flow of the solution. The temperature at which the movement of the meniscus on tilting of tube was arrested was recorded as gelation temperature and the time in seconds was recorded as gelation time.

### 2.4.2. Clarity

The clarity of the formulations before and after gelling was determined by visual examination against black and white background <sup>19</sup>.

### 2.4.3. pH

The pH of prepared formulations was determined at 25±2 °C by using calibrated digital pH meter (LI120 pH meter, ELICO Ltd). The sufficient quantity of prepared formulation was taken in a 50 ml beaker and the glass electrode was sufficiently dipped into the formulations <sup>4,13</sup>.

### 2.4.4. Gel strength

Gel strength was determined at gel state by graduated measuring jar and weight method <sup>8</sup>. The 50 g of gel was placed in a 100mL graduated measuring cylinder. The

weight equivalent to 35 g applied to the gel and the time taken by the weight to sink 5 cm down through the gel was noted. The gel strength was measured as the time (seconds, generally 25-50 sec) required to move the weight 5 cm down.

### 2.4.5. Drug content

The in situ gel formulation equivalent to 10mg of clozapine was taken and suitably diluted with appropriate solvent. The drug content was estimated by UV method as described earlier <sup>20</sup>.

### 2.4.6. Rheological properties at solution and gel states

The rheological properties of formulations at 25°C and 31°C were measured by using Brookfield DV-II + PRO viscometer with spindle no. 3 at 100 rpm. The prepared formulation was taken in a beaker and the viscosity was measured by immersing the spindle <sup>21,22</sup>.

### 2.4.7. Isolation of porcine nasal mucosa

The porcine nose was obtained from local slaughter house immediately after its sacrifice. It was transported to laboratory by keeping it in the phosphate buffer saline (pH 6.4). The intact nasal mucosa was separated from septum and the connective tissue using forceps and scissors without damaging the nasal mucosa and used within 30 min <sup>22</sup>.

### 2.4.8. Mucoadhesive strength

Mucoadhesive strength of gel state formulation was determined using modified physical balance <sup>4, 8, 21</sup>. The porcine nasal mucosa was used as biological membrane. Two cylindrical glass vials with 2.2 cm diameter and modified balance instrument were taken. The left side pan of the balance was removed and vial was fixed at base of it with cyanoacrylate glue and the other vial was attached to left arm of balance. The nasal mucosa was tied to one side of the both vials. The mucosa was hydrated with 100 μL of phosphate buffer saline pH 6.4. The gel formulation equivalent to 10mg of CZP was placed on lower vial prior to testing. Then, the vials were held together for 2 min for their attachment. After 2 min, weights were added to the right pan gradually until the mucosa was detached from each other. The total weight in grams required for the complete detachment of the mucosa was noted and the mucoadhesive strength in dyne/cm<sup>2</sup> was determined by using the following formula.

$$\text{Mucoadhesive Strength (dynes/cm}^2\text{)} = m \times g/A$$

Where **m** is weight required for detachment in grams, **g** is acceleration due to gravity (980 cm/s<sup>2</sup>) and **A** is surface area of mucosa exposed (3.8 cm<sup>2</sup>)

### 2.4.9. Ex vivo permeation of selected CZP formulations

Ex vivo drug permeation was studied as per the method described by Anuja Naik *et al*, 2014<sup>22</sup>. This study was carried out for selected gels and plain drug solution using Franz diffusion cell and porcine nasal mucosa. The freshly excised nasal mucosa was mounted on the Franz diffusion cell and clamped between the donor and receptor compartments and allowed to equilibrate for 30 min. The entire set up was placed on thermoregulatory magnetic stirrer and the temperature of the chamber was maintained at  $31 \pm 0.5^\circ\text{C}$  and stirred at  $250 \pm 2$  rpm. The formulation equivalent to 10 mg of CZP was placed in the donor compartment. The receiver compartment was filled with PBS pH 6.4. Samples (2mL) were withdrawn at the time intervals of 5, 15, 30, 60, 120, 240, 360 and 480 min. After each withdrawal, equal volume of fresh PBS pH 6.4 was replaced in the receptor compartment. The samples were filtered through  $0.2\mu\text{m}$  membrane filter, diluted and the absorbance was measured at 216 nm by using UV-Visible spectrophotometer. The cumulative amount of drug permeated at each time point was calculated by using following formula.

$$Q = [C_n V + \sum_{i=1}^{n-1} C_i S]$$

Where, Q= Cumulative amount of drug permeated;  $C_n$ = Concentration of drug ( $\mu\text{g/ml}$ ) in  $n^{\text{th}}$  sample interval; V= Volume of Franz diffusion cell,

$n-1$

$\sum C_i S$  = Sum of drug concentration of sample (1 to  $n-1$ ) multiplied with sampling volume (S)

### 2.4.10. Stability study

Stability study was carried out for optimized formulation (Fa15) at  $25^\circ\text{C} \pm 2$  and at  $4^\circ\text{C} \pm 2$  for 3 months<sup>8</sup>. Samples were withdrawn every month and analyzed for parameters like appearance, gelation temperature, viscosity, drug content, pH, ex vivo permeation of drug. After 3 months, the values of each observation were compared with the initial parameters.

## 3. RESULTS AND DISCUSSION

### 3.1. DSC thermogram of Clozapine

The DSC thermogram of clozapine showed a sharp endothermic peak at  $186.35^\circ\text{C}$  which corresponds to the melting point of clozapine which indicates crystalline and purity of drug. The thermogram was shown in Fig 1.

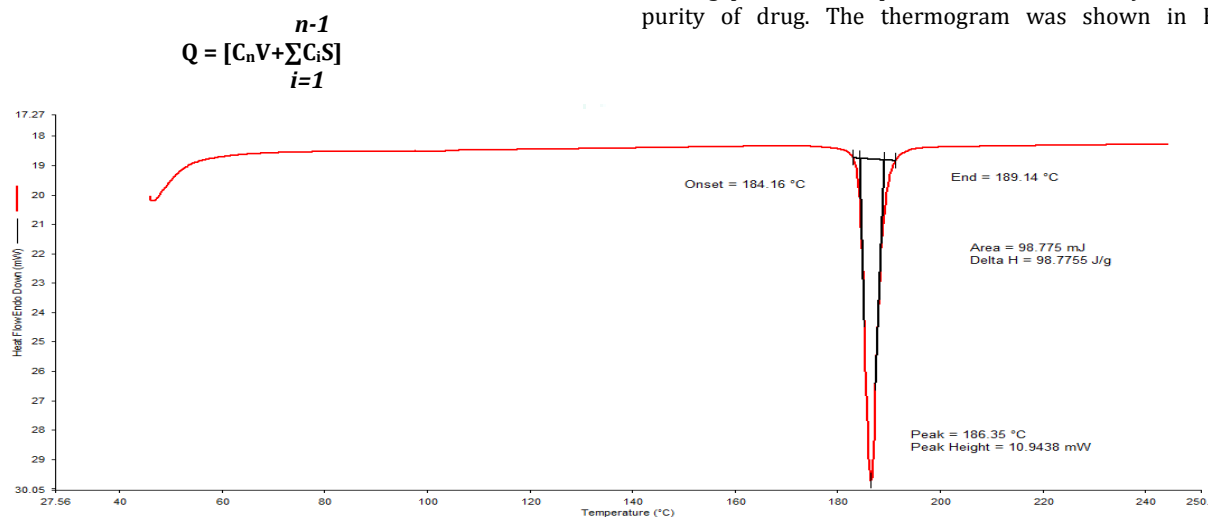


Figure 1: DSC thermogram of clozapine (CZP) pure drug

### 3.2. Solubility studies

The solubility of clozapine in various solvents was found to be in the range of  $0.014 \pm 0.001$  to  $155.54 \pm 2.72$  mg/ml (Fig 2). Based on CZP solubility, Transcutol P, Labrasol and Oleic acid were selected for development of CZP formulations.

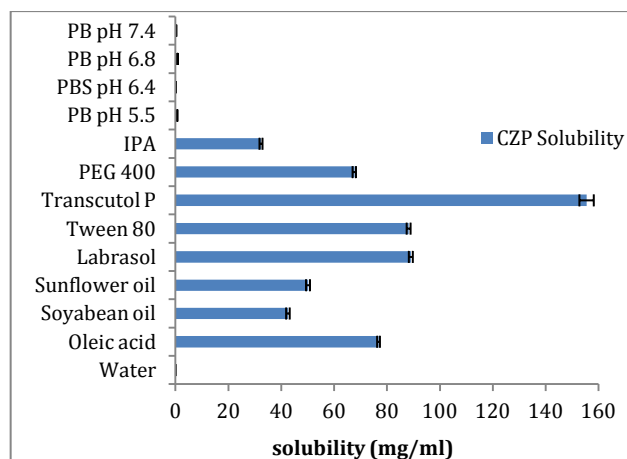


Figure 2: Solubility of clozapine in different solvents

PB: Phosphate buffer; PBS-Phosphate buffer saline; IPA-Isopropyl alcohol; PEG-Polyethylene glycol

### 3.3. Screening and optimization of composition

The optimization of pluronic concentration to achieve desired gelation temperature ( $T_{sol-gel}$ ) is a critical parameter during *in situ* gel development. Since the average temperature in human nose was reported to be  $31^\circ\text{C}$ . The nasal formulation should be converted to gel at nasal temperature (i.e.  $31-32^\circ\text{C}$ ) (Shuai Qian *et al* 2014, previously Jacky, 1980<sup>23</sup>; Proctor *et al*, 1977<sup>24</sup>). Therefore, the preliminary attempts were made to optimize the most suitable concentration of PF-127 to form a gel at a temperature about  $32^\circ\text{C}$ . At 20% concentration of PF-127 showed gelation temperature  $31.7 \pm 0.5^\circ\text{C}$  and hence selected for the formulation. PF-127 concentration showed inverse relation to the gelation temperature (Fig 3). The similar effect was reported by Preeti Pandey *et al*, 2017 and Shuai Qian *et al*, 2014. The gelation temperature increased as the PF-68 concentration increased (Fig 4). The formulation containing PF-127: PF-68 at 20:2 ratio showed optimal gelation temperature of  $31.7 \pm 0.5^\circ\text{C}$ .

Due to poor solubility of CZP, the required drug loading is not possible into a formulation (Yuan Yuan *et al*, 2012)<sup>25</sup>. This problem was solved by solubilising drug in Labrasol and Transcutol P mixture (1:1). The co solvent mixture Labrasol: Transcutol P (1:1) proportion was studied between 10-25% at fixed pluronic combination (Table 3). Increasing the concentration of surfactant, the gelation temperature also



increased which could be due to disruption of gel network by surfactant molecules (Tsutomu Furuya *et al*, 2003) <sup>26</sup>.

**Table 2: Gelation temperature and time of PF-127& PF-68 at different combinations**

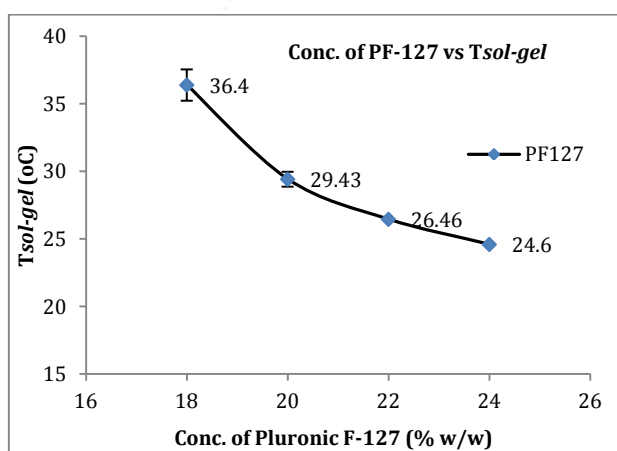
| PF-68 (%w/w) | PF-127 (%w/w) |          |               |          |               |          |               |          |
|--------------|---------------|----------|---------------|----------|---------------|----------|---------------|----------|
|              | 10            | 15       | 18            |          | 20            |          | 22            |          |
|              | Tsol-gel (°C) | GT (sec) | Tsol-gel (°C) | GT (sec) | Tsol-gel (°C) | GT (sec) | Tsol-gel (°C) | GT (sec) |
| 0            | No gelation   |          | 36.4±0.45     | 61±2     | 29.43±0.86    | 60±5     | 26.43±0.85    | 55±3     |
| 2            | No gelation   |          | 37.63±0.41    | 70±5     | 31.7±0.55     | 65±5     | 27.16±0.35    | 58±2     |
| 4            | No gelation   |          | 40.13±1.87    | 95±5     | 34.9±1.47     | 78±6     | 28.8±0.90     | 65±5     |
| 6            | No gelation   |          | 41.40±1.01    | 105±10   | 37.73±0.68    | 85±5     | 35.8±1.30     | 70±5     |
| 8            | No gelation   |          | 43.73±1.36    | 130±10   | 40.5±0.70     | 115±5    | 37.6±1.05     | 80±5     |

*Tsol-gel*-Gelation temperature from sol-gel; *GT*-Gelation time; *sec*-seconds; °C-degree Celsius; Each value represents the mean ± SD (n=3);

**Table 3: Effect of co solvent mixture on Tsol-gel of PF-127&PF-68(20:2)**

| L:Tp (%w/w) | Tsol-gel (°C) |
|-------------|---------------|
| 0           | 31.7±0.55     |
| 10          | 28.86±0.20    |
| 15          | 29.9±0.74     |
| 20          | 30.8±0.57     |
| 25          | 31.66±0.59    |

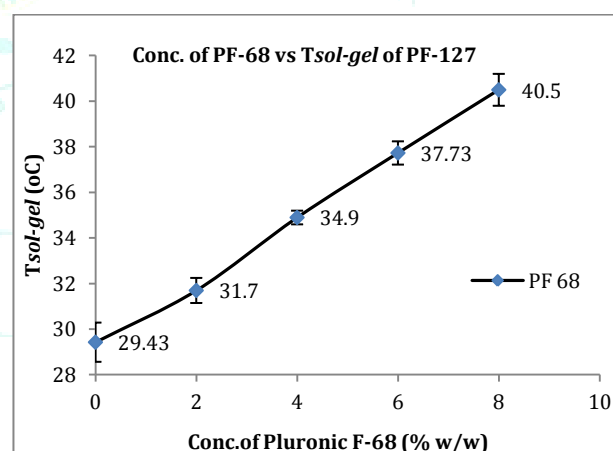
L: Tp: Labrasol: Transcutol P (1:1); Each value represents mean±SD (n=3)



**Figure 3: Effect of Pluronic F-127 on Tsol-gel**

### 3.4. Gelation Temperature (*Tsol-gel*) and Gelation time (*GT*) of prepared gels

The *Tsol-gel* of aqueous based gel formulations was in the range of 27.03±0.41 to 31.93±0.8 °C and oil based gel formulations was 26.86±0.4 to 30.53±0.66 °C (Table 4a & 4b). The effect of mucoadhesive agents such as Polyox WSR-303 a water soluble resin, Sodium-β-Glycerophosphate and 1 % w/w Chitosan (cationic polymer) was studied between the concentrations of 0.1% -0.5% w/w. The mucoadhesive polymers showed a concentration dependent *Tsol-gel* lowering effect (Fig 5a and Fig 5b). This could be due to the



**Figure 4: Effect of PF-68 on Tsol-gel at 20 % PF-127**

mucoadhesive agent bind to polyethylene oxide (PEO) chains present in the poloxamer molecules promoting dehydration and causing an increase in entanglement of adjacent molecules with more extensive intermolecular hydrogen bonding (Yingting Wang *et al*, 2017; Yuan Yuan *et al*, 2012; Zaki *et al.*, 2007<sup>27</sup>). The most pronounced decreasing effect was observed with Polyox WSR 303 as it produced high viscous formulation. The aqueous gel (Fa3, Fa10 and Fa15) and oil gel (Fo1, Fo9 and Fo13) formulations which exhibiting of gelation within 29–30°C were selected for further studies. All the formulations were transparent and the gelation occurred within 3min.

Table 4 a: Gelation temperature, Gelation time and % drug content of aqueous CZP nasal in situ gels

| F. code | Tsol-gel (°C) | GT (sec) | % Drug content |
|---------|---------------|----------|----------------|
| Fa0     | –             | –        | 99.97±0.01     |
| Fa1     | 31.66±1.48    | 90±1     | 99.53±0.49     |
| Fa2     | 30.36±0.90    | 82±2     | 98.67±1.07     |
| Fa3     | 29.43±0.47    | 78±2     | 99.84±0.12     |
| Fa4     | 27.9±0.50     | 70±1     | 99.53±0.47     |
| Fa5     | 27.03±0.41    | 65±1     | 99.62±0.32     |
| Fa6     | 31.93±0.80    | 126±2    | 99.46±0.62     |
| Fa7     | 31.03±1.09    | 102±10   | 98.74±0.85     |
| Fa8     | 30.66±0.51    | 90±5     | 99.15±0.64     |
| Fa9     | 30.26±0.45    | 81±6     | 99.42±0.45     |
| Fa10    | 29.86±0.65    | 95±5     | 99.75±0.20     |
| Fa11    | 31.83±0.51    | 102±7    | 99.9±0.06      |
| Fa12    | 30.46±0.35    | 94±4     | 99.92±0.03     |
| Fa13    | 30.23±0.45    | 84±2     | 99.72±0.16     |
| Fa14    | 30.00±1.94    | 73±2     | 99.44±0.68     |
| Fa15    | 29.6±1.70     | 69±5     | 99.95±0.02     |

Fa-Aqueous based formulation; Tsol-gel-Gelation temperature for sol-gel; GT-Gelation time

Note: All the formulations are yellowish, transparent & free flowing at 25°C and gelation at about 32°C

Table 4 b: Gelation temp, Gelation time and % drug content of oily CZP nasal in situ gels

| F. code | Tsol-gel (°C) | GT (sec) | % Drug content |
|---------|---------------|----------|----------------|
| Fo0     | –             | –        | 99.9±0.07      |
| Fo1     | 29.1±0.36     | 85±7     | 99.35±0.52     |
| Fo2     | 28.36±1       | 78±2     | 98.67±0.99     |
| Fo3     | 28.10±0.30    | 72±2     | 99.42±0.49     |
| Fo4     | 27.86±0.4     | 68±1     | 98.99±0.96     |
| Fo5     | 26.86±0.4     | 62±2     | 99.44±0.45     |
| Fo6     | 30.53±0.66    | 92±3     | 99.29±0.63     |
| Fo7     | 30.30±0.45    | 87±5     | 99.56±0.52     |
| Fo8     | 30.10±0.36    | 80±10    | 98.76±0.81     |
| Fo9     | 29.33±0.89    | 75±3     | 99.61±0.56     |
| Fo10    | 28.30±0.52    | 71±2     | 98.69±1.14     |
| Fo11    | 30.36±0.81    | 74±2     | 99.44±0.59     |
| Fo12    | 30.16±0.65    | 70±2     | 99.75±0.17     |
| Fo13    | 29.23±0.55    | 68±2     | 99.87±0.07     |
| Fo14    | 28.2±1.35     | 66±4     | 99.6±0.55      |
| Fo15    | 27.56±0.65    | 64±2     | 99.5±0.73      |

Fo-oil based formulations; Each value represents the mean ± SD (n=3);

Note: All the formulations are yellowish, transparent & viscous flowing at 25°C and gelation at about 31°C

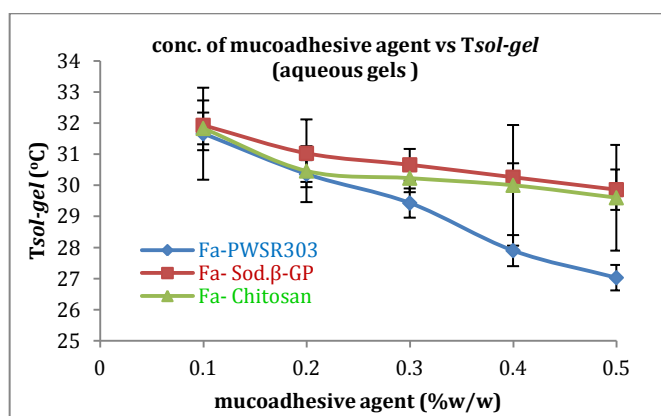


Figure 5a: Effect of mucoadhesive agent on Tsol-gel (Aqueous based gels)

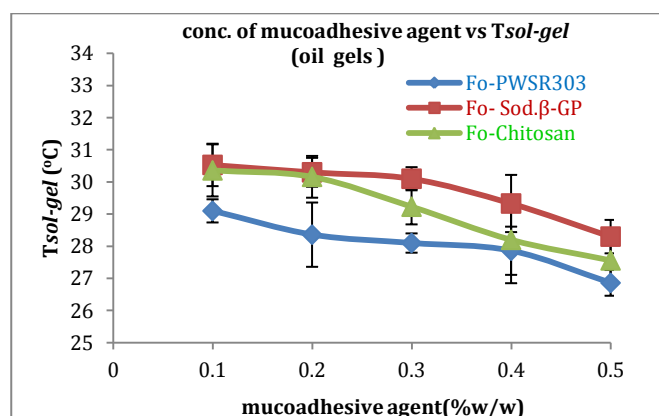


Figure 5b: Effect of mucoadhesive agent on Tsol-gel (oil based gels)

### 3.5. pH of formulations

The pH of the nasal formulation is important to avoid the irritation in the nasal cavity and it must be compatible with nasal secretions. In the present study, the pH of all formulation was adjusted to  $5.5 \pm 0.2$  with 0.1M HCl.

### 3.6. Drug content

The drug content of all the gel formulations, drug solutions were found in the range of  $98.67 \pm 0.99$ – $99.97 \pm 0.01\%$  and shown in tables 4a & 4b.

### 3.7. Gel strength

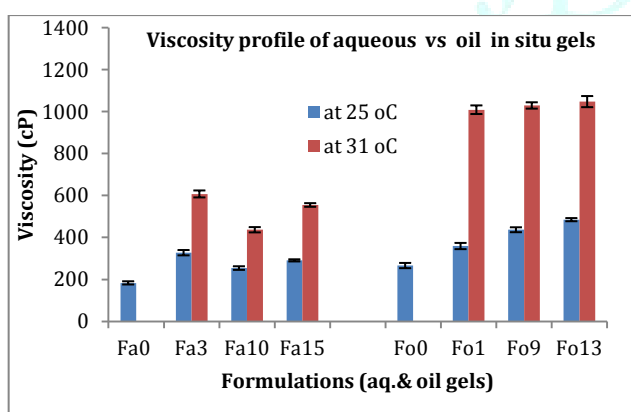
**Table 5: Tsol-gel, Gel strength and mucoadhesive strength of selected aqueous & oil gels**

| F. code             | Tsol-gel (°C)    | GS (sec)   | MS (dynes/cm <sup>2</sup> ) |
|---------------------|------------------|------------|-----------------------------|
| <b>Aqueous Gels</b> |                  |            |                             |
| Fa0                 | –                | –          | –                           |
| Fa3                 | $29.43 \pm 0.47$ | $67 \pm 7$ | $6060.52 \pm 128.94$        |
| Fa10                | $29.86 \pm 0.65$ | $35 \pm 5$ | $4169.29 \pm 74.44$         |
| Fa15                | $29.60 \pm 1.70$ | $45 \pm 5$ | $5114.91 \pm 107.37$        |
| <b>Oil Gels</b>     |                  |            |                             |
| Fo0                 | –                | –          | –                           |
| Fo1                 | $29.10 \pm 0.36$ | $75 \pm 5$ | $7307.01 \pm 196.97$        |
| Fo9                 | $29.33 \pm 0.89$ | $60 \pm 5$ | $5054.73 \pm 92.98$         |
| Fo13                | $29.23 \pm 0.55$ | $70 \pm 5$ | $6301.22 \pm 132.34$        |

**Fa0**-CZP plain solution (aqueous); **Fa3**-0.3% PWSR303; **Fa10**-0.5% Sod.β-GP; **Fa15**-0.5% Chitosan; **Fo0**-CZP plain solution (oil); **Fo1**-0.1% PWSR303; **Fo9**-0.4% Sod.β-GP; **Fo13**-0.3% Chitosan; **GS**-Gel Strength; **MS**-Mucoadhesive Strength; Each value represents the mean  $\pm$  SD (n=3)

### 3.8. Rheological properties of selected formulations (at solution state and gel state)

The viscosity of formulations was determined in solution state and gel state. The comparative profile between aqueous and an oil gels were shown in Fig 6. The higher viscosity values were obtained at 31°C and all formulations exhibited about 2 folds higher viscosity which indicated gelation of polymers at this temperature. The viscosity was proportional to the concentration of the mucoadhesive polymer in the formulation. The higher viscosity was produced by the formulations containing Polyox WSR303, followed by chitosan and sodium-β-glycerophosphate. The oil based gels showed extremely higher viscosities (~2 folds) than aqueous based gels. All the formulations had considerable flow at room temperature (25°C) whereas the flow was ceased at 31°C.



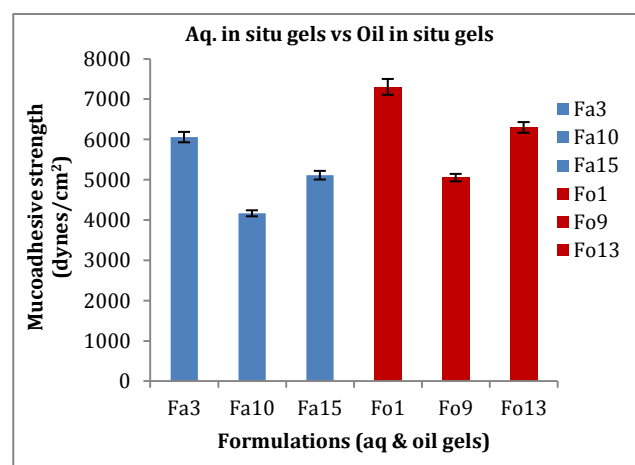
**Figure 6: Viscosity profile of selected *in situ* gels**

### 3.9. Mucoadhesive strength

The mucoadhesive strength is an important parameter for nasal formulations since it increases residence time in nasal cavity and prolonging the drug release (Hitendra S. Mahajan *et al*, 2011). However excessive mucoadhesive strength can damage the mucous membrane, thus an optimal strength is required for a nasal formulation (Preeti Pandey *et al*, 2017;

Gel strength was determined for selected formulations of aqueous and oil gels and results were shown in Table 5. It has been observed that gel strength was increased with the increase in the concentration of mucoadhesive polymer in the formulation. The oil based gel formulations showed higher gel strength than aqueous. The reason could be attributed to the oleic acid present in these formulations, as it has a tendency to increase the viscosity of the formulation. The gel strength values of both aqueous and oil formulations were between  $35 \pm 5$  sec and  $75 \pm 5$  sec. The formulations containing Polyox WSR 303 showed higher gel strength  $75 \pm 5$  sec (in oil gel) and  $67 \pm 7$  sec (in aqueous gel) than chitosan and sodium-β-glycerophosphate

Boddupalli *et al*, 2010<sup>28</sup>). The mucoadhesive strength between 4000-6000 dynes/cm<sup>2</sup> was considered adequate for a nasal formulation (Yingting Wang *et al*, 2017). The highest mucoadhesive strength was found with Polyox WSR 303 than chitosan and sodium-β-glycerophosphate in both types of gels. This is due to the fact that the formulations containing Polyox WSR 303 have high viscosity and chain flexibility which favours interaction with mucin. The lowest mucoadhesive strength was produced by sodium-β-glycerophosphate, whereas chitosan produced considerable mucoadhesion ( $5114.91 \pm 107.37$  dynes/cm<sup>2</sup>) in aqueous gels. The mucoadhesion of chitosan is due to the electrostatic attraction between the positively charged D-glucosamine units of chitosan and the negatively charged sialic acid and sulphate residues of mucin in nasal mucosa. The aqueous gels showed less mucoadhesive strength than oil gels due to the dilution effect of water and relatively weak cross-linked gel structure in aqueous gels. The decreasing order of mucoadhesive strength was observed as Polyox WSR 303>Chitosan>Sodium-β-GP. The comparative profile of mucoadhesive strength between aqueous and an oil gels was shown in Fig 7.



**Figure 7: Mucoadhesive strength of selected *in situ* gels**

### 3.10. Ex vivo permeation of selected CZP formulations

The ex vivo permeation studies carried out for selected formulations and the profiles of aqueous and oily gels were shown in Fig 8a and Fig 8b. The flux and permeability coefficients were calculated and compared with drug solution (Table 6). From the ex vivo permeation profiles, the permeation of drug was not only affected by pluronic concentration but also by the type of mucoadhesive agent used. All the formulations showed higher flux values when compared to drug solution. This could be due to the presence of Pluronic and cosolvents (L: Tp) which might also acts as permeation enhancers (Hitendra S. Mahajan *et al*, 2011; Parag M. Ved *et al*, 2011<sup>29</sup>). The formulations composed of Polyox WSR 303 showed less flux values when compared to chitosan and sodium- $\beta$ -GP due to.

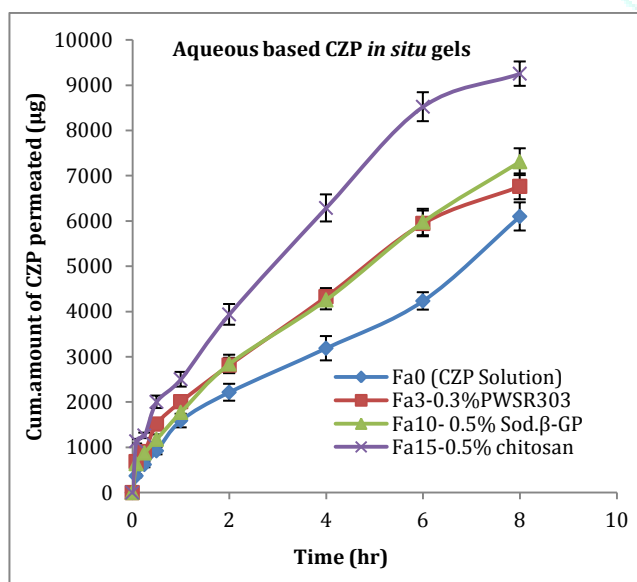
The flux values of oil based gels (136.47-190.34  $\mu\text{g}/\text{cm}^2/\text{h}$ ) were less than the aqueous based gels (159.89-243.46

$\mu\text{g}/\text{cm}^2/\text{h}$ ) due to their high viscosity and strong mucoadhesiveness. The high mucoadhesiveness can also retard the drug permeation (Yuan Yuan *et al*, 2012). The highest flux and permeability coefficients were obtained with formulations composed of chitosan when compared to others. This could be due to an interaction of a positively charged amino group on the C-2 position of chitosan with negatively charged sites on the cell membranes and tight junctions of the mucosal epithelial cells to allow opening of the tight junctions (Shagupfta khan *et al*, 2010). The increase in permeation shows contribution of paracellular transport through tight junctions. Therefore, the formulation Fa15, an aqueous based in situ gel composed of 0.5% chitosan showed highest flux (243.46  $\mu\text{g}/\text{cm}^2/\text{h}$ ) which was significantly high ( $p < 0.0001$ ) and 2.28 folds permeability enhancement than CZP plain drug solution. This greater permeability indicated that the entrapment of CZP in a hydrogel increases its diffusion.

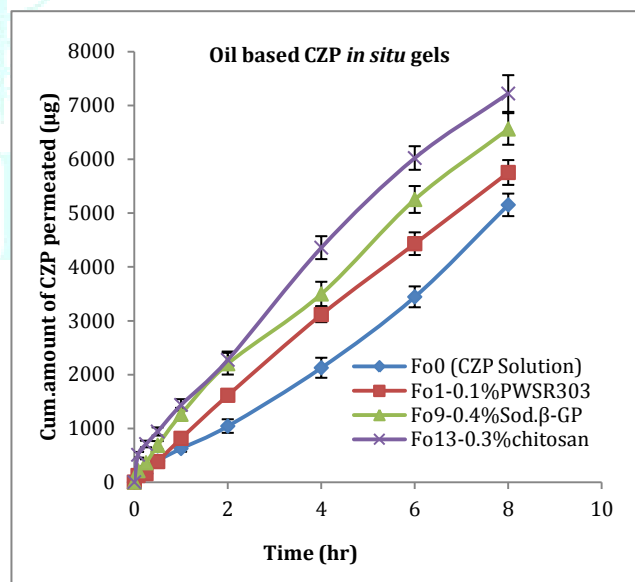
**Table 6: Flux, permeability coefficient and enhancement ratios of selected in situ gels**

| F.code              | Flux<br>( $\mu\text{g}/\text{cm}^2/\text{hr}$ ) | Kp x 10 <sup>-3</sup><br>(cm/hr) | ER   |
|---------------------|-------------------------------------------------|----------------------------------|------|
| <b>Aqueous Gels</b> |                                                 |                                  |      |
| Fa0                 | 106.34                                          | 10.63                            | 1    |
| Fa3                 | 159.89                                          | 15.98                            | 1.50 |
| Fa10                | 167.26                                          | 16.72                            | 1.57 |
| Fa15                | 243.46                                          | 24.34                            | 2.28 |
| <b>Oil Gels</b>     |                                                 |                                  |      |
| Fo0                 | 115.55                                          | 11.55                            | 1    |
| Fo1                 | 136.47                                          | 13.64                            | 1.18 |
| Fo9                 | 146.83                                          | 14.68                            | 1.27 |
| Fo13                | 190.34                                          | 19.03                            | 1.64 |

Kp- Permeability coefficient; ER-Enhancement ratio



**Figure 8a: Ex vivo permeation profile of CZP solution vs selected Aqueous *in situ* gels**



**Figure 8b: Ex vivo permeation profile of CZP solution vs selected oil *in situ* gels**



### 3.11. Stability studies for optimized (Fa15) formulation

The stability studies carried out for optimized aqueous based in situ gel formulation (Fa15) for 3 months. The formulation was found to be stable with no remarkable change in appearance, gelation properties, viscosity, mucoadhesiveness, drug content, pH and ex vivo drug permeation profiles (Table 7).

**Table 7: Stability studies for optimized formulation (Fa15)**

| Time period | Drug content (%) | Tsol-gel (°C) | Viscosity(cP) |              | MS (dynes/cm <sup>2</sup> ) | Cum.amt.drug Permeated (µg) | Flux (µg/cm <sup>2</sup> /h) | Kp    | ER   |
|-------------|------------------|---------------|---------------|--------------|-----------------------------|-----------------------------|------------------------------|-------|------|
|             |                  |               | at 25 °C      | at 31 °C     |                             |                             |                              |       |      |
| Initial     | 99.95±0.02       | 29.6±1.7      | 290.66±5.13   | 554.66±8.73  | 5114.91±107.37              | 9253.91±269.44              | 243.46                       | 24.34 | 2.28 |
| 1 month     |                  |               |               |              |                             |                             |                              |       |      |
| 25°C        | 99.92±0.02       | 29.5±0.1      | 291±8.18      | 555±13.22    | 5123.50±82.90               | 9224.40±371.48              | 243.26                       | 24.32 | 2.28 |
| 4°C         | 99.93±0.04       | 29.5±0.2      | 290±6.24      | 557±15.71    | 5132.1±92.98                | 9166.53±342.14              | 218.36                       | 21.83 | 2.05 |
| 2 month     |                  |               |               |              |                             |                             |                              |       |      |
| 25°C        | 99.91±0.02       | 29.4±0.1      | 292±6.55      | 558.33±10.40 | 5157.89±51.57               | 9116.82±332.97              | 242.24                       | 24.22 | 2.27 |
| 4°C         | 99.90±0.01       | 29.4±0.2      | 293.33±7.63   | 559±11.53    | 5140.70±53.68               | 9056.18±329.72              | 218.36                       | 21.83 | 2.05 |
| 3 month     |                  |               |               |              |                             |                             |                              |       |      |
| 25°C        | 99.89±0.01       | 29.2±0.3      | 294.66±6.42   | 560.33±9.5   | 5175.08±64.90               | 9045.11±336.92              | 241.22                       | 24.12 | 2.26 |
| 4°C         | 99.90±0.01       | 29.3±0.2      | 294.33±5.03   | 560.66±14.01 | 5166.49±39.39               | 9000.64±329.72              | 217.95                       | 21.79 | 2.04 |

Each value represents the mean ± SD (n=3)

**Note:** The appearance, pH of the formulation, Gelation time, Gelation strength were not changed significantly at entire period (not shown in table)

## CONCLUSIONS

The intranasal mucoadhesive aqueous and oil based *in situ* gels of clozapine were developed successfully by cold method using Pluronic F127 and F68 as thermosensitive gelling agents and Labrasol, Transcutol P as co solvents. The aqueous based in situ gels of CZP were superior than the oil based *in situ* gels which showed higher flux and enhancement ratio of 2.28. It can be concluded that the aqueous and oil based in situ gel formulation containing 0.5% chitosan showed significantly high permeation in ex vivo studies compared to others.

## ACKNOWLEDGEMENTS

The authors thanks to the University Grants Commission (UGC), New Delhi, India for the grant of Rajiv Gandhi National Fellowship to carry the research work.

## Conflict of Interests

The authors declare that they have no conflicts of interest to disclose

1. National Mental Health Programme, 2011; ICMR, India.
2. National Mental Health Survey of India .Prevalence, Pattern and Outcomes. MHFW, Govt. of India & NIMH&NS, 2015-16; 1-159.
3. Koppam M and Vobalaboina V. Pharmacokinetics, tissue distribution and bioavailability of clozapine solid lipid nanoparticles after intravenous and intraduodenal administration. J controlled Rel, 2005; 107:215-228.
4. Ravi PR, Aditya N, Patil S, Cherian L. Nasal in-situ gels for delivery of rasagiline mesylate: improvement in bioavailability and brain localization. Drug Delivery, 2013; 20(8):1-10.
5. Lisbeth Illum. Nasal Drug Delivery- Recent development and future prospects. J. Controlled Rel. 2012; 161(2):254-63.
6. YeZhan. Enhancing Antidepressant Effect of Poloxamer/Chitosan Thermosensitive Gel Containing Curcumin-Cyclodextrin Inclusion Complex. Int J Polymer Sci, 2018; 1-8.
7. Prabhjot K, Tarun G, Goutam R, Amit K.G. In situ nasal gel drug delivery: A novel approach for brain targeting through the mucosal membrane. Artificial Cells, Nanomed, and Bio tech, 2016; 44(4):1167-1176.
8. Upendra CG, Amruta BK, and Pravin DC. Development of in situ gel for nasal delivery: design, optimization, in vitro and in vivo evaluation. Drug Deliv, 2014; 21(1):62-73.
9. Hitendra SM, Saurabh KS, Sanjay JS. Nasal in situ gel containing hydroxy propyl b-cyclodextrin inclusion complex of artemether: development and in vitro evaluation. J Incl Phenom Macrocycl Chem, 2011; 70:49-58.
10. Shyamoshree B and Amal Kumar B. Development and Characterization of Mucoadhesive In Situ Nasal Gel of Midazolam Prepared with Ficus carica Mucilage. AAPS PharmSciTech, 2010; 11(3):1223-1231.
11. Parmar V, A.N.L. Development and evaluation of ion-dependent in- situ nasal gelling systems of metoclopramide hydrochloride as an antimigraine model drug. Int. J L Res in Sci and Tech, 2012; 1(2):80-89.
12. Juilee AK and Amelia MA. Pharmacodynamic and pharmacokinetic investigation of cyclodextrin mediated asenapine maleate in situ nasal gel for improved bioavailability, DDIP, 2017; 43(2):234-245.
13. Binu C and Surajpal V. Preparation and evaluation of novel in situ gels containing Acyclovir for the treatment of oral herpes simplex virus infections. The Sci World Journal, 2014; 1-7.
14. Yingting W, Shulong J, Hongli W, Haiyan B . A mucoadhesive, thermoreversible in situ nasal gel of geniposide for neurodegenerative diseases. PLoS ONE, 2017; 12 (12):1-17.
15. Schmolka, I.R. Artificial skin I. Preparation and properties of pluronic F-127 gels for treatment of burns. J Biomed Mat Res, 1972; 6:571- 582.
16. Preeti P, Peter J.C, Benjamin W, Benedict J.P., Harendra S.P. Formulation, functional evaluation and ex vivo performance of thermoresponsive soluble gels - A platform for therapeutic delivery to mucosal sinus tissue. Eur J Pharma Sci, 2017; 96:499-507.
17. Khan S, Patil K Yeole P, Gaikwad R. Brain targeting studies on buspirone hydrochloride after intranasal administration of mucoadhesive formulation in rats. J Pharm Pharmacol. 2009; 61(5):669-75.
18. Shuai Q, Yin Cheong W, Zhong Z. Development, characterization and application of *in situ* gel systems for intranasal delivery of tacrine. Int J Pharmaceutics, 2014; 468 (1-2):272-282.
19. Himanshu G, Aarti S, Birendra S. Pluronic and Chitosan based *in situ* gel system for periodontal application, Asian J of Pharmaceutics, 2009; 94-96.
20. Muhammad U, Sadaf I, Sadullah M, Abida KK, Rehana R. Formulation and in vitro evaluation of carbopol 934-based modified clotrimazole gel for topical application. Anais da Academia Brasileira de Ciências, 2016; 88(4):2303-2317.
21. Animesh Kumar, Tarun G, Ganti SS, Goutam R, Amit Kumar G. Optimization of combinational intranasal drug delivery system for the management of migraine by using statistical design. Eur J Pharm Sci, 2015; 70:140-151.
22. Anuja N and Hema N. Formulation and evaluation of thermosensitive biogels for nose to brain delivery of doxepin. Bio Med Res Int, 2014; 18:1-10.
23. Jacky and J.P. Barometric measurement of tidal volume: effects of pattern and nasal temperature. J App Physiology, 1980; 49:319-325.
24. Proctor, D.F., Andersen, I., Lundqvist, G.R. Human nasal mucosal function at controlled temperatures. Respiration Physiology, 1977; 30:109-124.
25. Yuan Y, Cui Y, Zhang L, Zhu H, Guo Y, Zhong B, Hu X, Zhang L, Wang X, Chen L. Thermosensitive and mucoadhesive in situ gel based on poloxamer as new carrier for rectal administration of Nimesulide. Int J Pharmaceutics, 2012; 430:114- 119.
26. Tsutomu F, Tsuyoshi K, Fumihiko T. Effects of Added Surfactants on Thermoreversible Gelation of Associating Polymer Solutions Journal of Polymer Science: Part B: Polymer Physics, 2003; 42:733-751.
27. Zaki, N.M., Awad, G.A., Mortada, N.D., Abd ElHady, S.S. Enhanced bioavailability of metoclopramide HCl by intranasal administration of a mucoadhesive in situ gel with modulated rheological and mucociliary transport properties. Eur.J. Pharm. Sci. 2007; 32:296-307.
28. Boddupalli, B.M., Mohammed, Z.N.K., Nath, R.A., Banji, D. Mucoadhesive drug delivery system: an overview. J. Adv. Pharm. Technol. Res, 2010; 1:381-387.
29. Parag M. Ved, Kwonho Kim. Poly(ethylene oxide/propylene oxide) copolymer thermo-reversible gelling system for the enhancement of intranasal zidovudine delivery to the brain, Int.J.Pharmaceutics,2011; 411:1-9.
30. Clozapine Data Sheet, Novartis Pharmaceuticals Ltd.
31. <http://www.drugbank.ca/drugs>