**RESEARCH ARTICLE**

**NANOEMULSION BASED INTRANASAL DELIVERY OF RISPERIDONE FOR NOSE TO BRAIN TARGETING**

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Risperidone nanoemulsion using different mucoadhesive agent as nasal drug delivery system was prepared to produce quick effect as compared to that of oral route. Solubility of drug was determined in different vehicles. Pseudo ternary phase diagram were generated using Acrysol K 150 as oil, tween 80 as a co-surfactant, and caproyl PGMC as a surfactant. The four formulations were prepared by the spontaneous emulsification method and were further characterized for their percentage transmittance, droplet size and zeta potential. Ex vivo diffusion study of the optimized batch was carried out using goat nasal mucosa. Histopathological study of the optimized batch was studied. Optimized formulation was found to possess the mean globule size 149 nm and zeta potential -17.3 mV. Ex vivo study revealed that at the end of 4 h, 93.76% of the dose was diffused successfully. In histopathological study, formulation treated mucosa did not show any damage to the epithelium layer.

**Key words:** Risperidone, Nanoemulsion, Spontaneous emulsification, Nasal ciliotoxicity.

**INTRODUCTION**

One of the major psychotic disorders is a schizophrenia that frequently has devastating effects on various aspects of the patient’s life and carries a high risk of suicide and other life threatening behaviors. The primary goal in management of schizophrenia is to achieve optimal control of symptoms (Nasrallah et al 2005). Antipsychotics are a group of powerful psychoactive drugs thought to block specific receptors in the brain that affect the central nervous system. A number of strategies are followed to target various body tissue/organs. The brain is a delicate organ with many vital functions, and formidable mechanisms isolate and protect it from the outside world. Unfortunately, the same mechanisms that prevent intrusive environmental chemicals accessing the brain also prevent the access of therapeutic chemicals (Soni et al 2004). The need for treatment options has been emphasized by the recent Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) study, in which 74% of 1493 patients with schizophrenia discontinued study medication within 18 months. The reasons for discontinuation included lack of efficacy, intolerability, and patient decision. The CATIE study has increased awareness of the need for new treatment options tailored to the choice of the individual patient and clinician (Canuso et al 2008).

There is a need of a therapeutic prompt action to rapidly control agitation and disturbed behaviors in patients with schizophrenia, make Risperidone (RPD), a possible candidate for the development of an intranasal formulation. RPD, 3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidiny] ethyl] - 6,7,8,9-tetrahydro-2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one, is an approved antipsychotic drug belonging to the chemical class of benzisoxazole derivative and is available as tablet, oral liquid (Risperidal®) and orally disintegrating tablet (Risperidal® M-TAB) (DrugBank, DB00734). Orally disintegrating tablet of RPD exhibit low bioavailability due to extensive first-pass
metabolism. The nontargeted delivery results in numerous side effects. Intramuscular route can be painful and cause a patient already in psychotic, fragile state of mind to feel even more insecure and increase their sense of being attacked (Wermeling and Miller, 2006). Target site of the RPD is brain, and direct transport of drugs to brain across the brain barriers following intranasal (i.n.) administration that provides a unique feature and better option for targeting drugs to brain (Behl et al 1998). The olfactory region of nasal mucosa provides a direct connection between nose and brain that can be exploited for targeting central nervous system (Kumar et al 2008). Mucoadhesive product provides a rapid transport of drug across nasal mucosa and a longer residence time in nasal cavity to overcome the nasal mucociliary clearance (Talegaonkar and Mishra, 2004; Kumar et al 2008). Nanoemulsion has already been utilized for intranasal as well as transdermal drug delivery (Talegaonkar et al 2011; Chouksey et al 2011; Mishra et al 2013). Keeping in view the potential of nanoemulsions (NEs) and target site of RPD in brain, present work was undertaken to investigate the feasibility of nose to brain delivery of RPD by developing its nanoemulsions formulation.

MATERIALS AND METHODS
Risperidone was obtained from the IPCA pharmaceutical, Mumbai as gift sample. Acrysol EL 135 and Acrysol K 150 gifted from the corel chemical, Ahmedabad. Caproyl PGMC and Labrafac PG obtained from Gattefosse France as gift sample. Acconon C-30, Captex 200 and Captex 1000 obtained from Abitech US. Tween 80 and Tween 60 purchased from Finar Chemical Ahmedabad.

Solubility study
Solubility of RPD was determined in various oils, surfactant and co-surfactants. One ml of each component was taken in screw cap vials with known quantity (130 mg) of excess drug. After sealing, vials were kept on water bath incubator shaker (Hicon Instrument, India) at 40±2°C for 48 h. After equilibrium each test tube was centrifuged (Remi, India) at 2500 rpm for 15 min. Solution was appropriately diluted with methanol and UV absorbance was measured at 279 nm against blank (methanol and same amount of oil present in test sample). Concentration of dissolved drug was determined using standard equation.

Construction of pseudo-ternary phase diagram
Pseudo ternary phase diagrams were developed using the aqueous titration method. Slow titration with the aqueous phase was performed for each combination of oil and Smix separately. The amount of aqueous phase added was varied to produce a water concentration in the range of 5% to 95% of total volume at around 5% intervals. Surfactant (Caproyl PGMC) and co-surfactant (Tween 80) were mixed (Smix) in different volume ratios (1:1, 1:2, 1:3, 2:1, 3:1). For each phase diagram, oil (Acrysol K 150) and specific Smix ratio were mixed thoroughly in different volume ratios from 1:9 to 9:1 in different glass vials. After each 5% addition of the aqueous phase to the oil: Smix mixture, visual observation was made. Similar method was used to develop pseudo ternary phase diagram for other ratio, a separate phase diagram was constructed using Chemix (Chemix school version 3.60 evaluation copy) software based on the observations noted (Agrawal et al 2012).

Nasal dose calculation of risperidone for human
For the nasal route dose of risperidone for the rat is equivalent to 0.09 mg/kg of body weight (Kumar et al 2008) On the bases of body surface area (BSA) human equivalent dose (HED) can be calculated by two way.

a) With the help of $K_m$ factor (Reagan-Shaw et al 2007)
Formula for dose translation based on BSA:
HED (mg/kg) = Animal dose (mg/kg) × Animal $K_m$ / Human $K_m$
Rat $K_m$ = 6, Human adult = 37,
Dose (rat) = 0.09 mg/kg
So, HED = 0.014 mg

b) With the help of body weight
HED = animal dose in mg/kg x (animal weight in kg/human weight in kg)$^{0.33}$
HED = 0.09 x (0.225/60)$^{0.33}$
= 0.09 x (0.00375)$^{0.33}$
= 0.014 mg
Oral dose of Risperidone = 1-3 mg
Nasal dose of Risperidone = 14 μg

**Formulation of nanoemulsion**

**Formulation of drug solution**
The Risperidone solution was prepared by dissolving RSP (35 mg) in 1 ml ethanol (95%, v/v) and finally volume was made to 10 ml with distilled water resulting in a solution of 3.5 mg/ml.

**Preparation of NE**
The NEs for Risperidone were prepared by the spontaneous emulsification method (titration method). The calculated amount of drug was added to the oily phase of NEs and magnetically stirred until dissolved followed by the addition of S_{mix} in a fixed proportion to produce a clear mixture. Then, a definite proportion of water was added and stirred to produce clear NE of Risperidone (Table 1).

**Table 1. Composition of batch S1 to S6**

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Composition (% v/v)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acrysol K 150</td>
<td>Caproyl PGMC</td>
</tr>
<tr>
<td>S1</td>
<td>14</td>
<td>30</td>
</tr>
<tr>
<td>S2</td>
<td>14</td>
<td>29</td>
</tr>
<tr>
<td>S3</td>
<td>14</td>
<td>28</td>
</tr>
<tr>
<td>S4</td>
<td>12</td>
<td>30</td>
</tr>
<tr>
<td>S5</td>
<td>12</td>
<td>29</td>
</tr>
<tr>
<td>S6</td>
<td>12</td>
<td>28</td>
</tr>
</tbody>
</table>

**Primary characterization**
Transmittance (%T) (Unit converter) : The percentage transmittance of each of 2 ml NEs was checked against distilled water using UV-VIS spectrophotometer at (Shimadzu-1800, Kyoto, Japan) 650 nm.

**Calculation of concentration of drug with the help of dropper**
From the prepared NE, 15 drops of formulation were taken in 50 ml of methanol than UV spectrophotometric analysis was carried out. Then, concentration of solution was calculated from the calibration curve and with help of the nasal dose, required concentration of the drug is calculated.

**Preparation of nanoemulsion with different formulation with different mucoadhesive agent**
The composition of nanoemulsion with different mucoadhesive agents are shown in Table 2.

**Table 2. Composition of nanoemulsion formulations**

<table>
<thead>
<tr>
<th>Drug solution*</th>
<th>S7 (ml)</th>
<th>S8 (ml)</th>
<th>S9 (ml)</th>
<th>S10 (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrysol K 150</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Caproyl PGMC</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Tween 80</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Chitosan</td>
<td>-</td>
<td>0.4%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carbopol 940</td>
<td>-</td>
<td>-</td>
<td>0.5%</td>
<td>-</td>
</tr>
<tr>
<td>Sodium alginate</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.5%</td>
</tr>
<tr>
<td>Water</td>
<td>43</td>
<td>43</td>
<td>43</td>
<td>43</td>
</tr>
</tbody>
</table>

**Characterization of nanoemulsion**

**Qualitative tests**
The dilution test was performed by diluting 1 ml of prepared NE(s) to 100 ml and observed for clarity/turbidity. To identify type of NE, methyl orange, a water soluble dye, was sprinkled over the NEs and observed microscopically. Centrifugation test, prepared NE(s) were centrifuged for 15 min and examined for whether the system was monophasic or biphasic.

**Drug content**
RSP from NE formulation was extracted in methanol. The solutions were filtered using whatman filter paper and the methanolic extract was analyzed for the RPD content by UV-visible spectrophotometer at 279 nm.

**Droplet size Analysis and (ζ)-zeta potential measurement**
After ensuring the complete dispersion of the
formulation, the droplet size and zeta potential of resultant nanoemulsion was determined by Zetasizer Nano Series (Microtrac Zeatrac, Canada).

**pH measurement**
The pH of the NEs was measured using pH meter (Model EQ 610, Equiptronics, India) using 5 ml sample in a 10 ml beaker.

**Conductivity measurement**
The conductivity of the NEs was measured with Conductometer (Model CL 220, Chemi line, India) by inserting the probe in 10 ml of prepared NE sample in a beaker.

**Viscosity measurement**
The viscosity of 20 ml of the sample was determined using a Brookfield viscometer (Model LVDF-II+P, USA) coupled with a Spindle T-B (entry code 92) at 50 rpm at 30°C.

**Ex vivo diffusion study of RSP formulation**
The freshly excised goat nasal mucosa, except the septum part, was collected from the slaughter house in phosphate buffer saline (PBS), pH 6.4 (Basu and Maity, 2012). The membrane was kept in PBS (pH 6.4) for 15 min to equilibrate. The superior nasal conche was identified and separated from the nasal membrane. The excised superior nasal membrane was then mounted on Franz diffusion cell. The tissue was stabilized using phosphate buffer (pH 6.4) in both the compartments and allowed to stir for 15 min on a magnetic stirrer. After 15 min, solution from both the compartments was removed and fresh phosphate buffer (pH 5) was filled in the acceptor compartment. The mounting of the nasal membrane was done using glue at the brim of the donor compartment to avoid leakage of the test sample and supported with thread crossing over the cell. The temperature of the receiver chamber containing 30 ml of diffusion media (phosphate buffer, pH 5.0) was controlled at 37°C under continuous stirring with Teflon coated magnetic bar at a constant rate, in a way that the nasal membrane surface just flushes the diffusion fluid. A drop of optimized Nanoemulsion formulation was placed in the donor compartment of Franz diffusion cell along with 1.7 ml of phosphate buffer (pH 5) added additionally. Samples from the receptor compartment were withdrawn at predetermined time intervals and analyzed. Each sample removed was replaced by an equal volume of diffusion media. Each study was carried for a period of 4.0 h, during which the drug in receiver chamber (µg/ml) across the goat nasal membrane was calculated at each sampling point. The cumulative values for % drug diffused were plotted against time.

**Histopathological study**
Freshly excised goat nasal mucosa, except for the septum, was collected from the slaughter house in saline phosphate buffer (pH 6.4). Three goat nasal mucosa pieces (X1, X2, and X3) with uniform thickness were selected and placed in glass petriplate. X1 was treated with 0.5 ml of PBS (pH 6.4, negative control), X2 with 0.5 ml of isopropyl alcohol (positive control), and X3 was treated with NE for 1 h. After 1 h, the mucosa were rinsed with PBS (pH 6.4) and subjected to histological studies to evaluate the toxicities of NEs and photographed by microscope. This test was performed at QUALITECH lab, Ahmedabad.

**Short term stability study**
The NE formulation was subjected to short term stability study under the following conditions: 40±5°C temperatures and 75±5% relative humidity in stability chamber (Thermolab, Bombay). The stability study was followed for 1 month. The stored sample was evaluated for appearance, color, pH, viscosity, conductivity and RSP content.

**RESULTS AND DISCUSSION**

**Solubility study of risperidone in various excipients**
Before selecting suitable excipients for risperidone nanoemulsion, UV spectrophotometric analysis of drug in each individual excipient was done by scanning the methanol drug excipients mixture in the range of 200-400 nm. It is expected that absence of any interference between the drug and the excipients, the absorption maxima of the drug remain intact even in its dissolved state in the said excipients. In this study, excipients were explored for solubility of Risperidone. For each excipient, λmax of the drug in methanol i.e. 279 nm was found to be retained. This information indicates that each of these excipients is well compatible with the drug at room temperature. The important criteria for selection of the excipients are that all the components are pharmaceutically acceptable and fall under GRAS (Generally regarded as safe).
category. For solubility studies various oils, surfactant and co-surfactants were selected. The solubility data of risperidone in various oil and surfactant are shown in Figure 1.

![Figure 1](image1.png)

**Figure 1.** Solubility data of risperidone in various oil and surfactant

Amongst all the tested materials, the maximum solubility of risperidone was found in the Caproyl PGMC (116.30±1.42 mg/ml), Acrysol K 150 (30.30±0.73mg/ml) and Tween 80 (20.44±0.76 mg/ml). Solubility of Risperidone in other oils ranges from 7 to 30 mg/ml. Among oils tested, Risperidone showed maximum solubility in Acrysol K 150, that’s why selected as the oil for the NE formulation. Among various surfactants tested, Risperidone showed maximum solubility in Caproyl PGMC (116.30±1.42 mg/ml) and Tween 80 (20.44±0.76 mg/ml). So, Caproyl PGMC selected as a surfactant and Tween 80 as a co-surfactant.

**Pseudo ternary phase diagram**

A ternary phase diagram was generated to choose the proper concentration of excipients i.e., oil proportion and optimum Smix ratio in the formulation to produce emulsions with good stability (the darken area in Figure 2).

![Figure 2](image2.png)

**Figure 2.** Pseudo ternary phase diagrams

As per pseudo ternary phase diagrams, it was concluded that the best surfactant mixture ratio is 2:1 this phase diagram contain the maximum region for the nanoemulsion formation compare to other Smix ratio.

**Primary characterization**

The optically clarity of batches S1 to S4 are shown in Table 3.

<table>
<thead>
<tr>
<th>Batch</th>
<th>Absorbance</th>
<th>% Transmittance</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>0.0082</td>
<td>98.13</td>
</tr>
<tr>
<td>S2</td>
<td>0.0062</td>
<td>98.58</td>
</tr>
<tr>
<td>S3</td>
<td>0.0073</td>
<td>98.33</td>
</tr>
<tr>
<td>S4</td>
<td>0.0048</td>
<td>98.90</td>
</tr>
<tr>
<td>S5</td>
<td>0.0063</td>
<td>98.56</td>
</tr>
<tr>
<td>S6</td>
<td>0.0077</td>
<td>98.23</td>
</tr>
</tbody>
</table>

From the data we can conclude that S4 batch is optically more clear.

**Calculation of concentration of drug with the help of dropper**

From the NE 15 drops of formulation were taken in 50 ml of methanol than UV analysis was carried out.

Concentration of solution = 10.81 µg/ml (from the calibration curve)

15 drops = 540.5 µg \(10.81 \times 50\) therefore, 1 drop = 36.03 µg

If the concentration of the NE is 0.35 mg/ml then, 1 drop contain the 36.03 µg of the drug.

Required dose of risperidone for nasal route = 14 µg

So, if we prepare NE with the concentration of 0.136 mg/ml than 1 drop of NE contains the 14 µg of the drug.

**Characterization of nanoemulsion**

**Qualitative test**

In general, an emulsion exhibits the characteristics of its external phase. Dilution tests are based on the fact that the emulsion is only miscible with the liquid that forms the continuous phase. Upon dilution, the emulsion retained its clarity indicating to be an o/w type of NE.

Staining tests in which a water-soluble dye is sprinkled onto the surface of the emulsion also indicate the nature of continuous phase. With an o/w emulsion, there is rapid incorporation of the
dye into the system, where as with w/o emulsion, the dye forms microscopically visible lumps. Methyl orange, a water soluble dye, could be readily incorporated in the NE system without clumping, hence proving the system to be of o/w type. Neither phase separation nor creaming on centrifugation of the NEs indicated stability of the prepared system. Evaluation parameters of batch S7 to S10 are shown in the Table 4.

<table>
<thead>
<tr>
<th>Batch</th>
<th>% Drug content*</th>
<th>Average droplet size (nm)</th>
<th>pH*</th>
<th>Conductivity (mS/cm)*</th>
<th>Zeta potential (mV)</th>
<th>Viscosity (cp)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>S7</td>
<td>98.71 ± 0.81</td>
<td>148.7</td>
<td>5.61 ± 0.12</td>
<td>0.139 ± 0.009</td>
<td>-15.3</td>
<td>237.5 ± 4.2</td>
</tr>
<tr>
<td>S8</td>
<td>99.32 ± 0.09</td>
<td>6000</td>
<td>5.36 ± 0.16</td>
<td>0.171 ± 0.01</td>
<td>-14.2</td>
<td>248.6 ± 1.6</td>
</tr>
<tr>
<td>S9</td>
<td>99.56 ± 0.24</td>
<td>6000</td>
<td>7.4 ± 0.18</td>
<td>0.133 ± 0.012</td>
<td>-15.96</td>
<td>332.5 ± 3.7</td>
</tr>
<tr>
<td>S10</td>
<td>99.49 ± 0.41</td>
<td>149.8</td>
<td>6.16 ± 0.13</td>
<td>0.202 ± 0.004</td>
<td>-17.3</td>
<td>635.1 ± 5.4</td>
</tr>
</tbody>
</table>

*Mean ± S.D, n=3

**Drug content**
Despite of difference in composition, the drug content of formulations S7 to S10 was found in range of 98.71-99.56 %.

**Droplet size determination**
The droplet size of the emulsion is a crucial factor in the rate and extent of drug release as well as absorption. S7 and S10 formulation passes the limit which is stated for the nanoemulsion particle (20 to 200 nm) while the S8 and S9 fails to pass. The increase in particle size may be related to the solubilization property of mucoadhesive agent incorporated.

**Zeta potential (ζ) determination**
ζ-potential can be defined as the difference in potential between surface of the tightly bound layer (shear plane) and the electro neutral region of an emulsion. It has got practical application in the stability of emulsion since, ζ-potential governs the degree of repulsion between adjacent, similarly charged, dispersed droplets. If the ζ-potential is reduced below a certain value (which is depends on a particular system being used), the attractive forces exceed the repulsive forces, and the particles come together leading to flocculation. In general, the zeta potential value of ±30 mV is sufficient for the stability of a nanoemulsion. But here as all the excipients used are non-ionic in nature, low zeta potential could be attributed to the drug molecule.

**pH measurement**
The pH of all the NEs ranged between 5.61 and 7.4. The normal pH range of nasal fluids is 4.5 to 6.5, which is one of the formulation considerations that might help reducing the irritation produced upon instillation. Batch S9 possessed the pH near to the neutral pH because carbopel solubilized at neutral pH. Eventhough many researcher used carbopel 940 in nasal formulation.

**Conductivity measurement**
Conductivity measurements rely on the poor conductivity of oil compared with water and give low values in water–oil emulsions where oil was the continuous phase. The reverse happened for o/w emulsion. The conductivity measurements (0.133–0.202 mS/cm) indicated the NEs to be of o/w type.

**Viscosity measurement**
For the longer residence time in the nasal membrane it is obvious to have moderately high viscosity. Formulations have the viscosity in the range of 237 to 635 cp. Batch S10 showed the highest viscosity among all other batches and hence considered better than other.

**Ex vivo diffusion study**
Nanoemulsion formulation was subjected to ex vivo permeation studies using the goat nasal mucosa. Ex vivo nasal mucosa diffusion profile was shown in Figure 3. The drug diffused at faster rate from nanoemulsion. The total percentage diffusion was much higher from the nanoemulsion system. The percent drug permeated after 4 h was found to be 93.76% from nanoemulsion.

**Histopathological study**
The microscopic observations (Figure 4) indicated that the optimized formulation did not significantly affect the microscopic structure of mucosa. As shown in Figure 4, neither cell
necrosis nor removal of the epithelium from the nasal mucosa was observed after permeation study of S10. Thus, NE formulations seemed to be safe with respect to nasal administration.

**Short term stability study**
Optimized formulation S10 was kept for the stability study for the one month period. The samples were withdrawn after one month and analysed for all basic evaluation tests and compared with result of initial time. There was no significant difference in drug content, pH, viscosity and conductivity at zero time and throughout the 1 month stability study period under all storage conditions. This indicated that risperidone is chemically and physically stable in the formulation and proved that the drug did not degrade or precipitate.

**CONCLUSION**
It was concluded that nasal nanoemulsion of risperidone could be successfully prepared by the spontaneous emulsification method by using the optimum concentration of 12% (v/v) of Acrosyl K 150, 30% (v/v) of Caproyl PGMC, 15% (v/v) Tween 80, 0.5% (w/v) Sodium alginate and 43% (v/v) of water, which can provide rapid transport of risperidone to brain via nasal mucosa by increasing the residence time in the nasal cavity. The prepared NE was free from nasal ciliotoxicity, narrow particle size distribution, excellent percentage transmittance, moderate Viscosity, suitable conductivity and pH and found stable for one month.

**REFERENCES**


