CHAPTER IV

4.1: FORMULATION OF ROPINIROLE HYDROCHLORIDE MICROEMULSION FOR INTRANASAL DELIVERY

4.1.1 OBJECTIVE

The present study is planned with the following objectives:

1. To study the Preformulation studies in order to investigate the interactions between the drug and polymer.
   a) Drug Characterization (Determination of Melting Point)
   b) Standard calibration curve
   c) Determination of Solubility of Ropinirole Hcl in Different Solvents
   d) Pseudo Ternary Phase Diagram
   e) Selection of Excipients (Oil, Surfactant, Co-Surfactants)
   f) FTIR

2. To design Ropinirole Hcl loaded Microemulsion formulation for brain targeting by Sonication Method using different selection of oil phase, Surfactants and Co-surfactants.

3. To perform the Physicochemical Characterization of the prepared microemulsion.
   a) Thermo dynamic Stability Studies
   b) Particle Size
   c) Poly Dispersability Index
   d) Viscosity Determination
   e) Refractive Index
   f) pH
   g) Zeta Potential
   h) Determination of Drug Content
   i) Scanning Electron Microscopy (SEM)

4. To carry out in vitro drug release studies and to explore the release behavior using various kinetic models.

5. To determine the In-vivo permeation of the optimized microspheres using mucus membranes of suitable animals adopting suitable methods.

6. To carry out stability studies for selected formulations as per ICH guidelines.
4.1.2 MATERIALS AND EQUIPMENTS

The following materials of pharma grade or the best possible. Laboratory Reagent (LR) were used as supplied by the manufacturer. The double distilled water was used in all experiments.

Table 2: List of Chemicals used with Grade and Manufacturer

<table>
<thead>
<tr>
<th>Material Used</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ropinirole Hcl</td>
<td>Hetero pharma, Hyderabad</td>
</tr>
<tr>
<td>Castrol oil</td>
<td>S.D.Fine Chemicals, Mumbai</td>
</tr>
<tr>
<td>Oleic Acid</td>
<td>S.D.Fine Chemicals, Mumbai</td>
</tr>
<tr>
<td>Sun flower oil</td>
<td>S.D.Fine Chemicals, Mumbai</td>
</tr>
<tr>
<td>Sesame Oil</td>
<td>S.D.Fine Chemicals, Mumbai</td>
</tr>
<tr>
<td>Tween 80</td>
<td>Oxford Chemicals, Mumbai</td>
</tr>
<tr>
<td>Tween 20</td>
<td>Oxford Chemicals, Mumbai</td>
</tr>
<tr>
<td>Span 20</td>
<td>Oxford Chemicals, Mumbai</td>
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<tr>
<td>Span 80</td>
<td>Oxford Chemicals, Mumbai</td>
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<tr>
<td>Menthol</td>
<td>Oxford Chemicals, Mumbai</td>
</tr>
<tr>
<td>PEG 400</td>
<td>Oxford Chemicals, Mumbai</td>
</tr>
<tr>
<td>Glycerin</td>
<td>S.D.Fine Chemicals, Mumbai</td>
</tr>
</tbody>
</table>
Table 3: List of Instruments Used

<table>
<thead>
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<th>Equipment Used</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precision Balance</td>
<td>Contech CA</td>
</tr>
<tr>
<td>Sonicator</td>
<td>Matri, Pondicherry</td>
</tr>
<tr>
<td>Lab Stirrer</td>
<td>Remi Motors Ltd</td>
</tr>
<tr>
<td>Ultra Sonicator</td>
<td>Biologics Inc (3000), India</td>
</tr>
<tr>
<td>Centrifuge</td>
<td>Remi Motors Ltd</td>
</tr>
<tr>
<td>Brookfield Viscometer</td>
<td>Model RVTDV II, Brookfield Engineering Laboratories Inc, Stoughton, MA</td>
</tr>
<tr>
<td>Keshary Chien Diffusion cell</td>
<td>Matchless Traders, Hyderabad</td>
</tr>
<tr>
<td>USP Dissolution Apparatus (paddle)</td>
<td>Lab India (DS-8000)</td>
</tr>
<tr>
<td>Magnetic Stirrer</td>
<td>2MLH Magnetic Stirrer mfg, Remi Equipments Pvt. Ltd.</td>
</tr>
<tr>
<td>Glasswares</td>
<td>Borosilicate</td>
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<tr>
<td>UV Spectrophotometer (Double Beam)</td>
<td>Lab India UV+3000</td>
</tr>
<tr>
<td>Scanning Electron Microscopy</td>
<td>Horiba</td>
</tr>
<tr>
<td>Particle Size Analyzer</td>
<td>Zetasizer 1000HS</td>
</tr>
<tr>
<td>Digital pH meter</td>
<td>Global Electronics</td>
</tr>
</tbody>
</table>
4.1.3 DRUG PROFILE \cite{153,154}

**Drug** : Ropinirole Hydrochloride

**Chemical name** : 4-[2-(dopropylamino)ethyl]-1,3-dihydro-2H-indol-2-one mono hydrochloride.

**Brand Names/Synonyms** : Ropinirole(INN- Spanish), Ropinirole Hel; Ropinirole hydrochloride; Ropinirolum (INN- Latin)

**Empirical Formula** : $C_{16}H_{24}N_2O\cdot HCL$.  

**Molecular Weight** : 296.84 (260.38 as the free base).

**Structure**

![Figure 6: Structure of Ropinirole HCL](image)

**Class** : Dopamine agonist.

**MECHANISM OF ACTION:**

Ropinirole hydrochloride is a non-ergoline dopamine agonist with high relative specificity and full intrinsic activity at the D2 and D3 dopamine receptor subtypes, binding with higher affinity to D3 than to D2 or D4 receptor subtypes. Ropinirole has moderate *In Vitro* affinity for opioid receptors. But its MOA is unknown in treating parkinsons disease although it is believed to be due to stimulation of postsynaptic dopamine D2 type receptors with in the caudate-putamen in the brain.

**Appearance** : White to pale greenish – yellow powder.

**Melting Range** : $243^0$ to $250^0C$

**Solubility** : 133 mg/ml in water. In other solvents it is partially soluble.
**Ultraviolet Spectrum**: $\lambda_{\text{MAX}}$ 210-250nm

**Pharmacokinetic**: Ropinirole is rapidly absorbed after oral administration, reaching peak concentration in approximately 1-2 hours. Food does not affect the extent of absorption of ropinirole, although its $t_{\text{max}}$ is increased by 2.5 hrs and its $C_{\text{max}}$ is decreased by 25% when taken with high meal.

- **Bioavailability (%)**: 35
- **Protein Binding (%)**: >40%
- **Elimination half life (hrs)**: 4-6hrs
- **Routes of administration**: oral & nasal.

**Metabolism**: hepatic (extensive)

**Common Adverse Effects**: Nausea, dizziness, hallucination and postural hypotension.

**Dose**

- **Tablet**: The usual dose is 3-9 mg daily and has to be taken in three divide doses owing to short half life of the drug.

- **Emulsion**: The usual dose is not more than 1ml at a time.

**Uses for Ropinirole Hydrochloride**:

1. In the treatment of parkinsons disease.
2. For restless leg syndrome.

**Storage**: Protect from light & moisture.
4.1.4 EXCIPIENTS PROFILE

4.1.4.1 CASTOR OIL [155]

Nonproprietary Names
BP: Virgin Castor Oil
JP: Castor Oil
PhEur: Castor Oil, Virgin
USP: Castor Oil

Synonyms
EmCon CO; Lipovol CO; oleum ricini; ricini oleum virginale; ricinoleum; ricinus communis; ricinus oil; tangantangan.

Empirical Formula and Molecular Weight
Castor oil is a triglyceride of fatty acids. The fatty acid composition is approximately ricinoleic acid (87%); oleic acid (7%); linoleic acid (3%); palmitic acid (2%); stearic acid (1%) and trace amounts of dihydroxystearic acid.

Description
Castor oil is a clear, almost colorless or pale yellow-colored viscous oil. It has a slight odor and a taste that is initially bland but afterwards slightly acrid.

Structure

Figure 7: Structure of Castor Oil
Applications in Pharmaceutical Formulation or Technology

Castor oil is widely used in cosmetics, food products, and pharmaceutical formulations. In pharmaceutical formulations, castor oil is most commonly used in topical creams and ointments at concentrations of 5–12.5%. However, it is also used in oral tablet and capsule formulations, ophthalmic emulsions, and as a solvent in intramuscular injections.

Typical Properties

Autoignition temperature: 449°C

Boiling point: 313°C

Density: 0.955–0.968 g/cm³ at 25°C

Flash point: 229°C

Melting point: -12°C

Moisture content: <0.25%

Description

Castor oil is a clear, almost colorless or pale yellow-colored viscous oil. It has a slight odor and a taste that is initially bland but afterwards slightly acrid.

Method of Manufacture

Castor oil is the fixed oil obtained by cold-expression of the seeds of Ricinus communis Linne´ (Fam. Euphorbiaceae). No other substances are added to the oil.

Safety

Castor oil is used in cosmetics and foods and orally, parenterally, and topically in pharmaceutical formulations. It is generally regarded as a relatively nontoxic and nonirritant material when used as an excipient. Castor oil has been used therapeutically as a laxative and oral administration of large quantities may cause nausea, vomiting, colic, and severe purgation. It should not be given when intestinal obstruction is present.

Stability and Storage Conditions

Castor oil is stable and does not turn rancid unless subjected to excessive heat. On heating at 300°C for several hours, castor oil polymerizes and becomes soluble in mineral oil. When cooled to 0°C, it becomes more viscous. Castor oil should be stored at a temperature not exceeding 25°C in well-filled airtight containers protected from light.
4.1.4.2 OLEIC ACID\textsuperscript{[156]}

Nonproprietary Names
BP: Oleic Acid
PhEur: Oleic Acid

Synonyms
Acidum oleicum; Crodolene; Crossential 094; elaic acid; Emersol; Glycon; Groco; Hy-Phi; Industrene; Metaupon; Neo-Fat; cis-9-octadecenoic acid; 9,10-octadecenoic acid; oleinic acid; Priolene.

Formula: C\textsubscript{18}H\textsubscript{34}O\textsubscript{2}
Molar mass: 282.4614 g/mol
Density: 895 kg/m\textsuperscript{3}
Boiling point: 360 °C
Melting point: 13 to 14 °C (55 to 57 °F; 286 to 287 K)

Structure:

\begin{center}
\includegraphics[width=0.5\textwidth]{oleic_acid_structure.png}
\end{center}

**Figure 8: Structure of Oleic Acid**

Solubility in water: Insoluble
Solubility in Ethanol: Soluble

Pharmacology: Oleic Acid has been reported to penetrate the skin of rats. On histological examination, fluorescence from absorbed oleic acid was found in epidermal cell layers of skin removed from treated rats within 10 min of its application. The path of penetration was suggested to be via the hair follicles. Only minute amounts of oleic acid were visualized in the blood vessels throughout the experiment. Skin permeability was shown to increase with the lipophilic nature of a compound.

Physical Description: Oleic acid is a colorless to pale yellow liquid with a mild odor. Floats on water.

Functional Category: Emulsifying agent; skin penetrant

Safety: Oleic acid is used in oral and topical pharmaceutical formulations. In vitro tests have shown that oleic acid causes rupture of red blood cells (hemolysis), and intravenous injection or ingestion of a large quantity of oleic acid can therefore be
harmful. The effects of oleic acid on alveolar and buccal epithelial cells in vitro have also been studied; the in vitro and in vivo effects of oleic acid on rat skin have been reported.

**Stability and Storage Conditions**

On exposure to air, oleic acid gradually absorbs oxygen, darkens in color, and develops a more pronounced odor. At atmospheric pressure, it decomposes when heated at 80–100°C. Oleic acid should be stored in a well-filled, well-closed container.

4.1.4.3 **SUNFLOWER OIL** [157]

**Nonproprietary Names**

**BP:** Refined Sunflower Oil

**PhEur:** Sunflower Oil, Refined

**USP-NF:** Sunflower Oil

**Synonyms**

Helianthi annui oleum raffinatum; huile de tournesol; oleum helianthi; sunflowerseed oil.

**Description**

Sunflower oil occurs as a clear, light yellow-colored liquid with a bland, agreeable taste.

**Structure**

![Figure 9: Structure of Sunflower Oil](image)

**Method of Manufacture**

Sunflower oil is obtained from the fruits and seeds (achenes) of the sunflower, Helianthus annus (Compositae), by mechanical means or by extraction.

**Safety**
Sunflower oil is widely used in food products and on its own as an edible oil. It is also used extensively in cosmetics and topical pharmaceutical formulations, and is generally regarded as a relatively nontoxic and nonirritant material.

**Typical Properties**

- **Boiling point**: 40–608°C
- **Density**: 0.915–0.919 g/cm³
- **Hydroxyl value**: 14–16
- **Iodine number**: 125–140
- **Melting point**: 18°C

Solubility: Miscible with benzene, chloroform, carbon tetrachloride, diethyl ether, and light petroleum; practically insoluble in ethanol (95%) and water.

**Stability and Storage Conditions**

Sunflower oil should be stored in an airtight, well-filled container, protected from light. Stability may be improved by the addition of an antioxidant such as butylated hydroxytoluene.

#### 4.1.4.4 SESAME OIL [158]

**Nonproprietary Names**

- **BP**: Refined Sesame Oil
- **JP**: Sesame Oil
- **PhEur**: Sesame Oil, Refined
- **USP-NF**: Sesame Oil

**Synonyms**

Benne oil; gingelly oil; gingili oil; jinjili oil; Lipovol SES; sesame oleum raffinatum; teel oil.

**Description**

Refined sesame oil is a clear, pale-yellow colored liquid with a slight, pleasant odor and a bland taste. It solidifies to a soft mass at about -4°C.

**Applications in Pharmaceutical Formulation or Technology**

The major use of sesame oil in pharmaceutical formulations is as a solvent in the preparation of sustained-release intramuscular injections of steroids, such as estradiol valerate, hydroxyprogesterone caproate, testosterone enanthate, and nandrolone decanoate, or other oil-soluble drug substances, such as the decanoates or enanthate.
esters of fluphenazine. The disappearance of sesame oil from the injection site, following subcutaneous or intramuscular administration to pigs, has been reported to have a half-life of about 23 days. A sesame paste (tahini), composed of crushed sesame seeds in sesame oil, has been investigated as a novel suspending agent.

**Structure**

![Figure 10: Structure of Sesame Oil](image)

**Typical Properties**

- **Density**: 0.916–0.920 g/cm³
- **Flash point**: 338°C (open cup)
- **Freezing point**: -5°C

Solubility Insoluble in water; practically insoluble in ethanol (95%); miscible with carbon disulfide, chloroform, ether, hexane, and light petroleum.

**Method of Manufacture**

Sesame oil is obtained from the ripe seeds of one or more cultivated varieties of *Sesamum indicum* Linne´ (Fam. Pedaliaceae) by expression in a hydraulic press or by solvent extraction. The crude oil thus obtained is refined to obtain an oil suitable for food or pharmaceutical use. Improved color and odor may be obtained by further refining.

**Stability and Storage Conditions**

Sesame oil is more stable than most other fixed oils and does not readily become rancid; this has been attributed to the antioxidant effect of some of its characteristic constituents. The PhEur 6.3 permits the addition of a suitable antioxidant to sesame oil. Sesame oil may be sterilized by aseptic filtration or dry heat.

It has been reported that suitable conditions for the sterilization of injections containing sesame oil are a temperature of 170°C for 2 hours; it has been suggested that 150°C for 1 hour is inadequate. However, it has been demonstrated that dry heat sterilization of sesame oil at 150°C for 1 hour was sufficient to kill all added *Bacillus*
subtilis spores. Sesame oil should be stored in a well-filled, airtight, light resistant container, at a temperature not exceeding 40\(^\circ\)C.

**4.1.4.5 TWEEN 80** \(^{[159]}\)

**Chemical Name**: Tween 80  
**Molecular Formula**: C24H44O6  
**Molecular Weight**: 428.600006103516  
**Properties**

- **BP**: >100\(^\circ\)C  
- **Density**: 1.08g/ml at 20\(^\circ\)C  
- **Vapour Pressure**: <1mm Hg(20\(^\circ\)C)  
- **Refractive index**: n20/D 1.473  
- **Storage temp.**: Store at RT.  
- **Form**: viscous liquid  
- **Water solubility**: 5-10g/100mL at 23\(^\circ\)C  

![Figure 11: Structure of Tween 80](image)

**Use**  
Nonionic surfactant suggested for use is cosmetic formulations (o/w emulsifier, viscosity modifier)

**General description**: Amber-colored viscous liquid. PH (5% aqueous solution) 5-7. Faint odour & bitter taste.

**Air & Water Reactions**: Water soluble.

**Reactivity Profile**: Tween 80 is incompatible with strong alkalis & oxidizers.
4.1.4.6 TWEEN 20 \textsuperscript{[160]}

**Synonyms**: Polysorbate 20, PEG sorbitan monolaurate.

**Appearance**: Clear, yellow to yellow-green viscous liquid.

**B.P**: $>100^0\text{c.}$

**Structure**

![Figure 12: Structure of Tween 20](image)

**Brookfield Viscosity**: 370-430 cps.

**pH of 1% aqueous solution**: 5-7.

**Refractive index**: 1.46.

**Specific gravity**: 1.1

**HLB**: 16.7.

**Molecular weight**: 1225 daltons.

**Uses**

Tween 20 is a nonionic detergent widely used in biochemical applications.

Emulsifying agents for the preparation of stable oil-in-water emulsions.

Storage / Stability: Store at room temperature. Tween 20 is heat sensitive and will darken when exposed to elevated temperatures.

4.1.4.7 SPAN 20 \textsuperscript{[161]}

**Molecular Formula**: C$_{18}$H$_{34}$O$_6$

**Molecular Weight**: 346.46

**Structure**

![Figure 13: Structure of Span 20](image)
Property
Span 20 is amber to brown oily liquid. non-toxic and odorless. Span 20 is slightly soluble in isopropanol, tetracarp, xylene, cotton seed oil and mineral oil, slightly soluble in liquid paraffin, and insoluble in water, HLB=8.6.

Use:
Span 20 is mainly used in medicine, cosmetics, textiles etc. as water/oil type emulsifier, wetting agent and lubricant.

Packing:
200kg iron drum/plastic drum.
Handle with care, Span 20 should be stored in cool, dry and draughty place. Shelf life is 2 years. Then, Span 20 can still be used if qualified after re-check.

4.1.4.8 SPAN 80

Molecular Formula: C_{24}H_{44}O_{6}
Molecular Weight: 428.6

Property:
Span 80 is light yellow viscose oily liquid. Span 80 is insoluble in water and soluble in organic solvents. It is water/oil type emulsifier, which can be mixed with emulsifier S60 and emulsifier T60. HLB: 4.3

Structure

![Figure 14: Structure of Span 80](image)

Use:
Span 80 is used as emulsifier, solubilizer, stabilizer, softener, anti-static agent etc. suitable for medicine, cosmetics, textiles, paints etc.

Packing and Storage:
In 200kg iron drum/plastic drum.
Handle with care, Span 80 should be stored in cool, dry and draughty place. Shelf life is 2 years. Then, Span 80 can still be used if qualified after re-check.
4.1.4.9 METHANOL \[163\]

Methanol, also known as methyl alcohol among others, is a chemical with the formula CH\(_3\)OH. Methanol acquired the name "wood alcohol" because it was once produced chiefly as a byproduct of the destructive distillation of wood. Wikipedia

**Formula:** CH\(_3\)OH

**Structure**

![Figure 15: Structure of Methanol](image)

**Boiling point:** 64.7 °C

**Density:** 792 kg/m\(^3\)

**Molar mass:** 32.04 g/mol

**Melting point:** -97.6 °C

**Vapor pressure:** 13.02 kPa

**Classification:** Alcohol

**Description:**

Methanol is a colorless, flammable liquid used in the manufacture of FORMALDEHYDE and ACETIC ACID, in chemical synthesis, antifreeze, and as a solvent. Ingestion of methanol is toxic and may cause blindness. It is the simplest alcohol, and is a light, volatile, colourless, flammable, poisonous liquid with a distinctive odor that is somewhat milder and sweeter than ethanol.

**Use:**

Methanol is used as a solvent and as an intermediate in chemical synthesis. Component of deicing mixtures and preservatives. Toxic.

4.1.4.10 POLYETHYLENE GLYCOL (PEG 400) \[164\]

PEG 400 is a low-molecular-weight grade of polyethylene glycol. It is a clear, colorless, viscous liquid. Due in part to its low toxicity, PEG 400 is widely used in a variety of pharmaceutical formulations. Wikipedia

**Density:** 1.13 g/cm\(^3\)

**Formula:** C\(_{2n}\)H\(_{4n+2}\)O\(_{n+1}\), \(n=8.2\) to 9.1
Structure

**Figure 16: Structure of Polyethylene Glycol (Peg 400)**

- **Molar mass**: 380-420 g/mol
- **Viscosity**: 90.0 cSt at 25 °C, 7.3 cSt at 99 °C
- **Melting point**: 4 to 8 °C (39 to 46 °F; 277 to 281 K)
- **LD50 (median dose)**: 30 mL/kg, orally in rats
- **Flash point**: 238 °C (460 °F; 511 K)
- **Description**: Clear colourless liquid
- **Specific gravity**: 1.11-1.12
- **pH of 1% solution**: 4.5-7
- **Solubility**: Soluble in water and ethanol.

**Uses:**

In pharma industries it is used as base for formulations. It is also used as base for creams and emulsions. It is widely used as co-surfactant in the preparation of microemulsion.

**Storage**

Store at room temperature.

**4.1.4.11 GLYCERIN**

**Nonproprietary Names**

- **BP**: Glycerol
- **JP**: Concentrated Glycerin
- **PhEur**: Glycerol
- **USP**: Glycerin

**Synonyms**

Croderol; E422; glicerol; glycerine; glycerolum; Glycon G-100; Kemstrene; Optim; Pricerine; 1,2,3-propanetriol; trihydroxypropane glycerol.

**Chemical Name**

Propane-1,2,3-triol
Structure

Figure 17: Structure of Glycerin

Empirical Formula
C₃H₈O₃

Molecular Weight
92.09

Description
Glycerin is a clear, colorless, odorless, viscous, hygroscopic liquid; it has a sweet taste, approximately 0.6 times as sweet as sucrose.

Typical Properties

Boiling point: 290°C (with decomposition)

Density
1.2656 g/cm³ at 15°C;
1.2636 g/cm³ at 20°C;
1.2620 g/cm³ at 25°C.

Flash point: 176°C (open cup)

Melting point: 17.8°C

Method of Manufacture
Glycerin is mainly obtained from oils and fats as a by-product in the manufacture of soaps and fatty acids. It may also be obtained from natural sources by fermentation of, for example, sugar beet molasses in the presence of large quantities of sodium sulfite. Synthetically, glycerin may be prepared by the chlorination and saponification of propylene.

Stability and Storage Conditions
Glycerin is hygroscopic. Pure glycerin is not prone to oxidation by the atmosphere under ordinary storage conditions, but it decomposes on heating with the evolution of toxic acrolein. Mixtures of glycerin with water, ethanol (95%), and propylene glycol are chemically stable.
4.1.5 PSEUDO TERNARY PHASE DIAGRAM

On the basis of the solubility studies, a combination of Castrol oil was selected as the oil phase, Tween 80 and PEG 400 were selected as surfactant and co-surfactant, respectively. Distilled water was used as an aqueous phase. Surfactant and Co-surfactant (Smix) were mixed at different mass ratios (1:1, 1:2, 2:1, 1:3).

These ratios were chosen in increasing concentration of surfactant with respect to surfactant for a detailed study of the phase diagrams. For each phase diagram, oil and Smix at a specific ratio was mixed thoroughly at different mass ratios from 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 in different glass vials. Different combinations of oil and Smix, were made so that maximum ratios were covered for the study to delineate the boundaries of phases precisely formed in the phase diagrams.

Pseudo ternary phase diagrams of Oil, Smix and aqueous phase were developed using the aqueous titration method. Slow titration with aqueous phase was performed for each mass ratio of oil and Smix and visual observations were made for transparent and easily flowable o/w Microemulsions.

The physical state of the Microemulsion was marked on a pseudo-three component phase diagram with one axis representing the aqueous phase, the second one representing oil and the third representing a mixture of surfactant and co-surfactant at a fixed mass ratio.

Psuedo-ternary Phase Diagram was taken to ensure that observations were not made on metastable systems; although the free energy required to form an emulsion is very low, the formulation is thermodynamically spontaneous. The relationship between the phase behavior of a mixture and its composition can be captured with the aid of a phase diagram. Psuedoternary phase diagrams were constructed separately for each Smix ratio, so that o/w Microemulsion regions could be identified and Microemulsion formulations could be optimized. Psuedoternary phase diagrams were constructed separately for each Smix ratio as shown in figure which represents the Smix Ratio 1:1, 1:2, 2:1, 1:3 respectively. It was observed in 1:1 Smix that when cosurfactant was added along with surfactant, the interfacial film became more fluid and no liquid crystalline area was found in the phase diagram. A large o/w microemulsion area was observed. The maximum amount of oil that could be solubilized was 34% (m/m) with around 28% (m/m) of Smix. As the surfactant concentration was increased in Smix ratio (1:1), a higher microemulsion region was observed. It may be due to further
reduction of the interfacial tension, increasing the fluidity of the interface, thereby increasing the entropy of the system. There may be a greater penetration of the oil phase in the hydrophobic region of the surfactant monomers. As we further increased surfactant concentration in the Smix to 2:1, the microemulsion region decreased as compared to 1:1, the maximum concentration of oil that could be solubilized by this ratio was 36% (m/m) with around 31% (m/m) of Smix. When Smix ratio of 1:2 was studied the small area of microemulsion further decreased and the liquid crystalline area started to appear in the phase diagram, which may be due to increased surfactant concentration. The maximum concentration of the oil that could be solubilized by this ratio was 41% (m/m) with around 42% (m/m) of Smix. When Smix ratio of 3:1 was studied the small area of microemulsion further decreased and the liquid crystalline area started to appear in the phase diagram, which may be due to increased surfactant concentration. The maximum concentration of the oil that could be solubilized by this ratio was 42% (m/m) with around 34% (m/m) of Smix. When co surfactant concentration was increased from 1:1 to 1:2 compared to surfactant, the microemulsion area was decreased. It is well known that large amounts of surfactants cause skin irritation, it is therefore important to determine the surfactant concentration properly and use the optimum concentration of surfactant in the formulation. From psuedoternary phase diagrams, the formulation in which the amount of oil phase completely solubilized the drug and which could accommodate the optimum quantity of Smix and distilled water were selected for the study.

Figure 18: An example showing aqueous titration endpoint for construction of psuedo-ternary phase diagram
Figure 19: Psuedo Ternary phase diagram showing the o/w microemulsion (Shaded Area) regions of Castrol oil (oil), Tween 80 (Surfactant), PEG 400 (Cosurfactant) at Smix ratio 1:1

Figure 20: Psuedo Ternary phase diagram showing the o/w microemulsion(Shaded Area) regions of Castrol oil (oil), Tween 80 (Surfactant), PEG 400( Cosurfactant) at Smix ratio 1:2

Figure 21: Psuedo Ternary phase diagram showing the o/w microemulsion(Shaded Area) regions of Castrol oil (oil), Tween 80 (Surfactant), PEG 400( Cosurfactant) at Smix ratio 2:1

Figure 22: Psuedo Ternary phase diagram showing the o/w microemulsion(Shaded Area) regions of Castrol oil (oil), Tween 80 (Surfactant), PEG 400( Cosurfactant) at Smix ratio 1:3
Determination of Concentration Range of the Selected Formulation Components by Construction of Ternary Phase Diagram

A series of emulsifying formulations were prepared with varying concentrations of Oil, Smix and water. Concentration of Castrol oil was varied from 4-70% (w/w) as an oil phase, Smix from 40-8% (w/w) which includes both Tween 80 and PEG 400 and water concentration which is varied in a concentration of 56-22% (w/w). Total of the Oil, Smix and water added up to 100% in each mixture. Each formulation was homogenized with the heat up to 45-50°C. Those compositions having % transmittance more than 70%.

Table 4: % Transmittance for plotting ternary phase diagram

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Smix Ratio</th>
<th>% Transmittance</th>
<th>S.No.</th>
<th>Smix Ratio</th>
<th>% Transmittance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1</td>
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<td></td>
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<tr>
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<td>2:8</td>
<td>90.7</td>
<td>2</td>
<td>2:8</td>
<td>86.7</td>
</tr>
<tr>
<td>3</td>
<td>3:7</td>
<td>81.3</td>
<td>3</td>
<td>3:7</td>
<td>89.5</td>
</tr>
<tr>
<td>4</td>
<td>4:6</td>
<td>86.2</td>
<td>4</td>
<td>4:6</td>
<td>86.6</td>
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<tr>
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<td>5:5</td>
<td>82.8</td>
<td>5</td>
<td>5:5</td>
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<td>6:4</td>
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<td>7</td>
<td>7:3</td>
<td>75.8</td>
</tr>
<tr>
<td>8</td>
<td>8:2</td>
<td>88.3</td>
<td>8</td>
<td>8:2</td>
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</tr>
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</tr>
<tr>
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<td>1:2</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>1:9</td>
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<td>1:9</td>
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</tr>
<tr>
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<td>2:8</td>
<td>73.3</td>
<td>2</td>
<td>2:8</td>
<td>87.2</td>
</tr>
<tr>
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<td>3:7</td>
<td>83.8</td>
<td>3</td>
<td>3:7</td>
<td>85.3</td>
</tr>
<tr>
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<td>4:6</td>
<td>81.2</td>
<td>4</td>
<td>4:6</td>
<td>81.3</td>
</tr>
<tr>
<td>5</td>
<td>5:5</td>
<td>84.1</td>
<td>5</td>
<td>5:5</td>
<td>87.2</td>
</tr>
<tr>
<td>6</td>
<td>6:4</td>
<td>84.2</td>
<td>6</td>
<td>6:4</td>
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<td>7</td>
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<td>84.9</td>
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<tr>
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<td>81.8</td>
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<td>8:2</td>
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</tr>
<tr>
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<td>81.5</td>
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<td>9:1</td>
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</tr>
<tr>
<td>1:3</td>
<td></td>
<td></td>
<td>1:3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BHAGWANT UNIVERSITY
EXPERIMENTAL WORK

Figure 23: Ternary phase diagram showing the o/w microemulsion regions of Castrol oil (oil), Tween 80 (Surfactant), PEG 400 (Cosurfactant)

From the above ternary phase diagram the concentration ranges of Castrol oil, Smix and water can be obtained as shown in table.

Table 5: Concentration Ranges of Castrol oil (oil), Tween 80 (Surfactant), PEG 400 (Cosurfactant) and water

<table>
<thead>
<tr>
<th>S.No</th>
<th>Components</th>
<th>Concentration Ranges</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Castrol oil</td>
<td>4 - 70</td>
</tr>
<tr>
<td>2</td>
<td>Smix (Tween 80 and PEG 400)</td>
<td>40 - 8</td>
</tr>
<tr>
<td>3</td>
<td>Water</td>
<td>56 - 22</td>
</tr>
</tbody>
</table>

4.1.6 FORMULATION OF ROPINIROLE HCL MICROEMULSION

4.1.6.1 Selection of Formulations from Phase Diagram

Formulations were selected from the microemulsion region of the constructed phase diagram to incorporate drug into the oil phase. The formulation was chosen with the criteria of maximum oil being emulsified with the minimum amount of Smix. The constant amount of Ropinirole Hcl that is 1% w/w of Ropinirole Hcl, was selected for the formulations, was dissolved in the oil phase of the emulsion formulation. Selected Formulations were subjected to different thermodynamic stability tests.

4.1.6.2 Determination of Concentration Range of the selected formulation components by construction of Ternary Phase Diagrams

Construction of Ternary Phase diagram was aimed at determining the concentration range of each factor as well as to identify the desired region of emulsification possess clear emulsion characteristic. A series of emulsifying formulations were prepared with varying concentrations of Oil, Smix and water. Concentration of Castrol oil was varied from 4-70% (w/w) as an oil phase, Smix from 40-8% (w/w) which includes both Tween 80 and PEG 400 and water concentration which is varied in a concentration of 56-22% (w/w). Total of the Oil, Smix and water added up to 100% in each mixture. Each formulation was homogenized with the gentle heat up to 45-50°C. Accurately weighed 50mg of each mixture was then emulsified to 50 ml with
distilled water, under the conditions of gentle shaking and the resultant emulsion was allowed to stand undisturbed for 15min for equilibration. The selection of emulsification range was done on the virtual clearance and % Transmittance. Only those compositions having % transmittance more than 70% and clear appearance were considered desirable and were used in plotting ternary phase diagram. Ternary phase diagrams were plotted using Chemix Software (version 3.60). Desirable emulsifying region and concentration range of each component were identified from phase diagram. Observations are recorded in table - and the ternary phase diagram is plotted in figure. The three sides of the triangle represent Oil, Smix and Water the coloured area represents the desired emulsifying region.

4.1.6.3 Formulation Development

From the results of solubility, emulsifying ability, concentration ranges to be used from pseudo ternary phase diagram Castrol oil, Tween 80 and PEG 400 as oil, Surfactant and Co-Surfactant were weighed and mixed well. The drug was accurately weighed to represent 1% of the total weight of the formulation and added to the previous mixture and stirred with a magnetic bar on magnetic stirrer, at room temperature until the drug completely dissolved. The weighed amount of water then added dropwise with continuous mixing. Then droplet size is further reduced by sonication method.

4.1.6.4 Sonication Method [166]

In this method the droplet size of conventional emulsion are reduced with the help of sonication mechanism. Only small batches of Microemulsion can be prepared by this method.

<table>
<thead>
<tr>
<th>Table 6: Developed Formulas of Ropinirole HCL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Formulation Code</strong></td>
</tr>
<tr>
<td>-----------------------</td>
</tr>
<tr>
<td>F1</td>
</tr>
<tr>
<td>F2</td>
</tr>
<tr>
<td>F3</td>
</tr>
<tr>
<td>F4</td>
</tr>
<tr>
<td>F5</td>
</tr>
<tr>
<td>F6</td>
</tr>
<tr>
<td>F7</td>
</tr>
<tr>
<td>F8</td>
</tr>
<tr>
<td>F9</td>
</tr>
<tr>
<td>F10</td>
</tr>
</tbody>
</table>
4.1.6.5 Formula Optimization

From the diffusion profiles of the 10 developed formulations in different buffers which indicated the ability of the system to self-emulsify with minimum contact of aqueous phase, the formulations having highest diffusion rate as % release of drug vs Time (hours) in buffers are selected as optimized formulations for final evaluation.

Table 7: Composition of Selected microemulsion Formulations

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Oil/Smix Ratio</th>
<th>Oil (mg)</th>
<th>Smix (mg)</th>
<th>Water (Parts)</th>
<th>Oil (Parts)</th>
<th>Smix (Parts)</th>
<th>Water (Parts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>1:1</td>
<td>1000</td>
<td>9000</td>
<td>1250</td>
<td>4</td>
<td>40</td>
<td>56</td>
</tr>
<tr>
<td>F2</td>
<td>1:1</td>
<td>2000</td>
<td>8000</td>
<td>10700</td>
<td>10</td>
<td>39</td>
<td>51</td>
</tr>
<tr>
<td>F3</td>
<td>1:1</td>
<td>3000</td>
<td>7000</td>
<td>9350</td>
<td>16</td>
<td>36</td>
<td>48</td>
</tr>
<tr>
<td>F4</td>
<td>1:1</td>
<td>4000</td>
<td>6000</td>
<td>6850</td>
<td>24</td>
<td>35</td>
<td>41</td>
</tr>
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<td>F5</td>
<td>1:1</td>
<td>5000</td>
<td>5000</td>
<td>5650</td>
<td>32</td>
<td>32</td>
<td>36</td>
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<tr>
<td>F6</td>
<td>1:1</td>
<td>6000</td>
<td>4000</td>
<td>5200</td>
<td>40</td>
<td>26</td>
<td>34</td>
</tr>
<tr>
<td>F7</td>
<td>1:1</td>
<td>7000</td>
<td>3000</td>
<td>4500</td>
<td>48</td>
<td>21</td>
<td>31</td>
</tr>
<tr>
<td>F8</td>
<td>1:1</td>
<td>8000</td>
<td>2000</td>
<td>3400</td>
<td>60</td>
<td>15</td>
<td>25</td>
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<td>F9</td>
<td>1:1</td>
<td>9000</td>
<td>1000</td>
<td>2900</td>
<td>70</td>
<td>8</td>
<td>22</td>
</tr>
<tr>
<td>F10</td>
<td>1:2</td>
<td>1000</td>
<td>9000</td>
<td>11100</td>
<td>5</td>
<td>44</td>
<td>53</td>
</tr>
</tbody>
</table>

A total of 10 formulations were selected based on their ability to form oil in water microemulsions which are selected from pseudoternary phase diagram of each Smix as shown in the table 10.
4.2: FORMULATION DESIGN OF ROPINIROLE HYDROCHLORIDE MICROSPHERE FOR INTRANASAL DELIVERY

4.2.1 OBJECTIVE

The present study is planned with the following objectives:

- To conduct Preformulation studies in order to investigate the interactions between the drug and polymer.
  a) Drug Characterization (Determination of Melting Point)
  b) Standard calibration curve
  c) FTIR
- To design Ropinirole Hcl loaded nasal microspheres formulation for brain targeting by emulsion solvent evaporation technique using three polymers, that is, chitosan, carbopol 974P and guar gum individually and in combinations.
- To examine the properties of Ropinirole Hcl loaded microsphere formulations.
- To perform the Physicochemical Characterization of the prepared microspheres.
  a) Micromeritic properties of microspheres
  b) Percentage yield
  c) Particle size analysis
  d) Drug entrapment efficiency (DEE)
  e) Loose surface crystallography (LSC)
  f) Swelling index (SI)
- To carry out in vitro drug release studies and to explore the release behavior using various kinetic models.
- To determine the In-vivo permeation of the optimized microspheres using mucus membranes of suitable animals adopting suitable methods.
- To carry out stability studies for selected formulations as per ICH guidelines.
4.2.2 MATERIALS AND EQUIPMENTS

Table 8: List of Chemicals used and suppliers

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>MATERIALS</th>
<th>COMPANY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ropinirole Hydrochloride</td>
<td>Hetero pharma , Hyderabad</td>
</tr>
<tr>
<td>2</td>
<td>Chitosan</td>
<td>Finar chemicals limited, Ahmedabad</td>
</tr>
<tr>
<td>3</td>
<td>Guar gum</td>
<td>Burgoyne Burbridges &amp; co, Mumbai</td>
</tr>
<tr>
<td>4</td>
<td>Carbopol 974P</td>
<td>Burgoyne Burbridges &amp; co, Mumbai</td>
</tr>
<tr>
<td>5</td>
<td>Span 80</td>
<td>Finar chemicals limited, Ahmedabad</td>
</tr>
<tr>
<td>6</td>
<td>Tween 80</td>
<td>Finar chemicals limited, Ahmedabad</td>
</tr>
<tr>
<td>7</td>
<td>Light Liquid Paraffin</td>
<td>Molychem, Mumbai</td>
</tr>
<tr>
<td>8</td>
<td>n-Hexane</td>
<td>Finar chemicals limited, Ahmedabad</td>
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<tr>
<td>9</td>
<td>Pottasium dihydrogen phosphate</td>
<td>Qualigens fine chemicals, Bombay</td>
</tr>
<tr>
<td>10</td>
<td>Sodium Hydroxide pellets</td>
<td>Finar chemicals limited, Ahmedabad</td>
</tr>
<tr>
<td>11</td>
<td>Concentrated Hydrochloric acid</td>
<td>Molychem, Mumbai</td>
</tr>
</tbody>
</table>

Table 9: List of instruments used

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>INSTRUMENT</th>
<th>MANUFACTURER</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Magnetic Stirrer with Hot plate</td>
<td>Remi motors, Ahmedabad</td>
</tr>
<tr>
<td>2</td>
<td>Mechanical Stirrer</td>
<td>Remi motors, Ahmedabad</td>
</tr>
<tr>
<td>3</td>
<td>Hot Air Oven</td>
<td>Ever flow scientific instruments</td>
</tr>
<tr>
<td>4</td>
<td>Ultra Sonicator</td>
<td>Life care equipment pvt. ltd</td>
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<tr>
<td>5</td>
<td>Optical Microscopy</td>
<td>Kshitiz innovations</td>
</tr>
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<td>6</td>
<td>UV/ VIS Spectrophotometer</td>
<td>LAB INDIA UV 3000+</td>
</tr>
<tr>
<td>7</td>
<td>Dissolution apparatus</td>
<td>LAB INDIA DS 8000</td>
</tr>
<tr>
<td>8</td>
<td>FTIR 200 Spectrometer</td>
<td>Perkin Elmer</td>
</tr>
<tr>
<td>9</td>
<td>Scanning Electron Microscopy</td>
<td></td>
</tr>
</tbody>
</table>
4.2.3 DRUG PROFILE \cite{167,168}

Drug : Ropinirole Hydrochloride

Chemical name : 4-[2-(dopropylamino)ethyl]-1,3-dihydro-2H-indol-2-one mono hydrochloride.

Brand Names/Synonyms : Ropinirole(INN- Spanish), Ropinirole Hel; Ropinirole hydrochloride ; Ropinirolum (INN- Latin)

Empirical Formula : C_{16}H_{24}N_{2}O.HCL.

Molecular Weight : 296.84(260.38 as the free base).

Structure

![Figure 24: Structure of Ropinirole Hcl](image)

Class : Dopamine agonist.

MECHANISM OF ACTION:

Ropinirole hydrochloride is a non-ergoline dopamine agonist with high relative specificity and full intrinsic activity at the D2 and D3 dopamine receptor subtypes, binding with higher affinity to D3 than to D2 or D4 receptor subtypes. Ropinirole has moderate In Vitro affinity for opioid receptors. But its MOA is unknown in treating parkinsons disease although it is believed to be due to stimulation of postsynaptic dopamine D2 type receptors with in the caudate-putamen in the brain.

Appearance : White to pale greenish – yellow powder.

Melting Range : 243\textdegree to 250\textdegree C

Solubility : 133 mg/ml in water. In other solvents it is partially soluble.
Ultraviolet Spectrum: $\lambda_{\text{MAX}}$ 210-250 nm

**Pharmacokinetic:** Ropinirole is rapidly absorbed after oral administration, reaching peak concentration in approximately 1-2 hours. Food does not affect the extent of absorption of ropinirole, although its $t_{\text{max}}$ is increased by 2.5 hrs and its $C_{\text{max}}$ is decreased by 25% when taken with high meal.

- Bioavailability (%): 35
- Protein Binding (%): >40%
- Elimination half life (hrs): 4-6hrs
- Routes of administration: oral & nasal.
- **Metabolism:** hepatic (extensive)

**Common Adverse Effects:** Nausea, dizziness, hallucination and postural hypotension.

**Dose:**

**Tablet:** The usual dose is 3-9 mg daily and has to be taken in three divide doses owing to short half life of the drug.

**Emulsion:** The usual dose is not more than 1ml at a time.

**Uses for Ropinirole Hydrochloride:**

3. In the treatment of parkinsons disease.
4. For restless leg syndrome.

**Storage:** Protect from light & moisture.
4.2.4 EXCIPIENTS PROFILE

4.2.4.1 CHITOSAN [169]

Nonproprietary Names
BP: Chitosan Hydrochloride
PhEur: Chitosan Hydrochloride

Synonyms
2-Amino-2-deoxy-(1,4)-b-D-glucopyranan; chitosani hydrochloridum; deacetylated chitin; deacetylchitin; b-1,4-poly-D-glucosamine; poly-D-glucosamine; poly-(1,4-b-D-glucopyranosamine).

Chemical Name
Poly-b-(1,4)-2-Amino-2-deoxy-D-glucose

Empirical Formula and Molecular Weight
Chitosan is the term applied to deacetylated chitins in various stages of deacetylation and depolymerization and it is therefore not easily defined in terms of its exact chemical composition. A clear nomenclature with respect to the different degrees of N-deacetylation between chitin and Chitosan has not been defined and as such chitosan is not one chemical entity but varies in composition depending on the manufacturer. In essence, chitosan is chitin sufficiently deacetylated to form soluble amine salts. The degree of deacetylation necessary to obtain a soluble product must be greater than 80–85%. Chitosan is commercially available in several types and grades that vary in molecular weight by 10 000–1 000 000, and vary in degree of deacetylation and viscosity.

Description
Chitosan occurs as odorless, white or creamy-white powder or flakes. Fiber formation is quite common during precipitation and the chitosan may look ‘cottonlike’.

Structure

![Figure 25: Structure of Chitosan](image)
Safety
Chitosan is being investigated widely for use as an excipient in oral and other pharmaceutical formulations. It is also used in cosmetics. Chitosan is generally regarded as a nontoxic and nonirritant material. It is biocompatible with both healthy and infected skin. Chitosan has been shown to be biodegradable.
LD50 (mouse, oral): >16 g/kg

Typical Properties
Acidity/alkalinity pH = 4.0–6.0 (1% w/v aqueous solution)
Density 1.35–1.40 g/cm³
Glass transition temperature 203°C
Moisture content Chitosan adsorbs moisture from the atmosphere, the amount of water adsorbed depending upon the initial moisture content and the temperature and relative humidity of the surrounding air.
Particle size distribution <30 mm
Solubility Sparingly soluble in water; practically insoluble in ethanol (95%), other organic solvents, and neutral or alkali solutions at pH above approximately 6.5.

Stability and Storage Conditions
Chitosan powder is a stable material at room temperature, although it is hygroscopic after drying. Chitosan should be stored in a tightly closed container in a cool, dry place. The PhEur 6.5 specifies that chitosan should be stored at a temperature of 2–8°C.

4.2.4.2 GUAR GUM [170]

Nonproprietary Names
BP: Guar Galactomannan
PhEur: Guar Galactomannan
USP-NF: Guar Gum

Synonyms
E412; Galactosol; guar flour; guar galactomannanum; jaguar gum; Meyprogat; Meyprodor; Meyprofin.

Chemical Name
Galactomannan polysaccharide
Structure

Figure 26: Structure of Guar Gum

Description
The USP32–NF27 describes guar gum as a gum obtained from the ground endosperms of Cyamopsis tetragonolobus (L.) Taub. (Fam. Leguminosae). It consists chiefly of a high-molecular-weight hydrocolloidal polysaccharide, composed of galactan and mannan units combined through glycoside linkages, which may be described chemically as a galactomannan. The PhEur 6.3 similarly describes guar galactomannan as being obtained from the seeds of Cyamopsis tetragonolobus (L.) Taub. by grinding the endosperms and subsequent partial hydrolysis.

Typical Properties
Acidity/alkalinity pH = 5.0–7.0 (1% w/v aqueous dispersion)
Density 1.492 g/cm3
Solubility Practically insoluble in organic solvents. In cold or hot water, guar gum disperses and swells almost immediately to form a highly viscous, thixotropic sol. The optimum rate of hydration occurs at pH 7.5–9.0. Finely milled powders swell more rapidly and are more difficult to disperse. Two to four hours in water at room temperature are required to develop maximum viscosity.
Viscosity (dynamic) 4.86 Pa s (4860 cP) for a 1% w/v dispersion.

Method of Manufacture
Guar gum is obtained from the ground endosperm of the guar plant, Cyamopsis tetragonolobus (L.) Taub. (Fam. Leguminosae), which is grown in India, Pakistan, and the semiarid southwestern region of the USA.
The seed hull can be removed by grinding, after soaking in sulfuric acid or water, or by charring. The embryo (germ) is removed by differential grinding, since each
component possesses a different hardness. The separated endosperm, containing 80% galactomannan is then ground to different particle sizes depending upon final application.

Safety
Guar gum is widely used in foods, and oral and topical pharmaceutical formulations. Excessive consumption may cause gastrointestinal disturbance such as flatulence, diarrhea, or nausea. Therapeutically, daily oral doses of up to 25 g of guar gum have been administered to patients with diabetes mellitus.

Stability and Storage Conditions
Aqueous guar gum dispersions have a buffering action and are stable at pH 4.0–10.5. However, prolonged heating reduces the viscosity of dispersions. The bacteriological stability of guar gum dispersions may be improved by the addition of a mixture of 0.15% methylparaben and 0.02% propylparaben as a preservative. In food applications, benzoic acid, citric acid, sodium benzoate, or sorbic acid may be used. Guar gum powder should be stored in a well-closed container in a cool, dry place.

4.2.4.3 CARBOPOL 974P [171]
Chemical Formula: C₅H₁₀O₂
IUPAC Name: 2-methylbutanoic acid
Molecular Weight: 102.133 g/mol
Structure

![Figure 27: Structure of Carbopol 974P](image)

Description
It is a highly crosslinked polymer and produces highly viscous gels with rheology similar to mayonnaise. Drug release from extended release tablets is affected by differences in the rates of hydration and swelling of the polymer hydrogel, which are largely defined by the crosslinker levels.
4.2.4.4 SPAN 80\textsuperscript{[172]}

**Molecular Formula:** $C_{24}H_{44}O_6$

**Molecular Weight:** 428.6

**Property:**

Span 80 is light yellow viscose oily liquid. Span 80 is insoluble in water and soluble in organic solvents. It is water/oil type emulsifier, which can be mixed with emulsifier S60 and emulsifier T60. HLB: 4.3

**Structure**

![Structure of Span 80](image)

**Use:**

Span 80 is used as emulsifier, solubilizer, stabilizer, softener, anti-static agent etc. suitable for medicine, cosmetics, textiles, paints etc.

**Packing and Storage:**

In 200kg iron drum/plastic drum.

Handle with care, Span 80 should be stored in cool, dry and draughty place. Shelf life is 2 years. Then, Span 80 can still be used if qualified after re-check.

4.2.4.5 TWEEN 80\textsuperscript{[173]}

**Chemical Name:** Tween 80


**Molecular Formula:** $C_{24}H_{44}O_6$

**Molecular Weight:** 428.6

**Properties**

- **BP:** $>100^\circ$C
- **Density:** 1.08g/ml at 20$^\circ$C
- **Vapour Pressure:** <1mm Hg (20$^\circ$C)
- **Refractive index:** n20/D 1.473
- **Storage temp.:** Store at RT.
- **Form:** viscous liquid
Water solubility  : 5-10g/100mL at 23°C

Structure:

![Figure 29: Structure of Tween 80]

Use
Nonionic surfactant suggested for use is cosmetic formulations (o/w emulsifier, viscosity modifier)

**General description:** Amber-colored viscous liquid. PH (5% aqueous solution) 5-7. Faint odour & bitter taste.

**Air & Water Reactions:** Water soluble.

**Reactivity Profile:** Tween 80 is incompatible with strong alkalis & oxidizers.

4.2.4.6 LIGHT LIQUID PARAFFIN [174]

**Nonproprietary Names**
- **BP:** Light Liquid Paraffin
- **JP:** Light Liquid Paraffin
- **PhEur:** Paraffin, Light Liquid
- **USP-NF:** Light Mineral Oil

**Synonyms**
905 (mineral hydrocarbons); Citation; light liquid petrolatum; light white mineral oil; paraffinum perliquidum.

**Description**
Light mineral oil is a transparent, colorless liquid, without fluorescence in daylight. It is practically tasteless and odorless when cold, and has a faint odor when heated. The USP32–NF27 specifies that light mineral oil may contain a suitable stabilizer.
**Structure**

![Figure 30: Structure of Liquid Paraffin](image)

**Typical Properties**
Solubility Soluble in chloroform, ether, and hydrocarbons; sparingly soluble in ethanol (95%); practically insoluble in water.

**Safety**
Light mineral oil is used in applications similar to those of mineral oil. Mineral oil is considered safe by the FDA for direct use in foods. However, oral ingestion of large doses of light mineral oil or chronic consumption may be harmful. Chronic use may impair appetite and interfere with the absorption of fat-soluble vitamins.

**Stability and Storage Conditions**
Light mineral oil undergoes oxidation when exposed to heat and light. Oxidation begins with the formation of peroxides, exhibiting an ‘induction period’. Under typical storage conditions, the induction period may take months or years. However, once a trace of peroxide is formed, further oxidation is autocatalytic and proceeds very rapidly. Oxidation results in the formation of aldehydes and organic acids, which impart taste and odor.

The USP32–NF27 permits the addition of suitable stabilizers to retard oxidation, butylated hydroxyanisole, butylated hydroxytoluene, and alpha tocopherol being the most commonly used antioxidants. Light mineral oil may be sterilized by dry heat. Light mineral oil should be stored in an airtight container in a cool, dry place and protected from light.

4.2.4.7 n-HEXANE [175]

**Chemical formula:** $C_6H_{14}$

**Molar mass:** 86.18 g·mol$^{-1}$

**Appearance:** Colorless liquid

**Odor:** Petrolic
Density: 0.6548 g mL\(^{-1}\)

**Melting point:** −96 to −94 °C; −141 to −137 °F; 177 to 179 K

**Solubility in water:** 9.5 mg L\(^{-1}\)

**Viscosity:** 0.3 mPa·s

**Structure**

![Figure 31: Structure of n-Hexane](image)

**Description:** n-Hexane is a very volatile aliphatic hydrocarbon. It is a constituent in the paraffin fraction of crude oil and natural gas and is also used as an industrial chemical and laboratory reagent. Laboratory grade n-hexane contains approximately 99% n-hexane. “Hexane” or “hexanes” is a commercial and industrial product consisting of a mixture of hydrocarbons with six carbon atoms and includes n-hexane and its isomers 2-methylpentane and 3-methylpentane as well as small amounts of other hydrocarbons.

4.2.4.8 **POTASSIUM DIHYDROGEN PHOSPHATE** \(^{[176]}\)

**Chemical Names:** Potassium dihydrogen phosphate; 7778-77-0; MONOPOTASSIUM PHOSPHATE; Potassium phosphate monobasic; Phosphoric acid, monopotassium salt; Potassium phosphate,

**Molecular Formula:** KH\(_2\)PO\(_4\) or H\(_2\)KO\(_4\)P

**Molecular Weight:** 136.084 g/mol

**Structure**

![Figure 32: Structure of Potassium Dihydrogen Phosphate](image)

**Assay:** Not less than 98.0% after drying

**Description**

Odourless, colourless crystals or white granular or crystalline powder

**Characteristics Identification**

**Solubility:** Freely soluble in water; insoluble in ethanol

**pH:** 4.2 - 4.7
Purity

**Loss on drying:** Not more than 2% (105⁰, 4 h)

**Water insoluble substances:** Not more than 0.2%

**Fluoride:** Not more than 10 mg/kg

**Density:** 2.34 g/cu cm

**Method of Assay**

Transfer about 5 g of the dried sample, accurately weighed, into a 250-ml beaker. Add 100 ml of water and 5 ml of 1 N hydrochloric acid, and stir until the sample is completely dissolved. Place the electrodes of a suitable pH meter in the solution, and slowly titrate the excess acid, stirring constantly, with 1 N sodium hydroxide to the inflection point occurring at about pH 4. Record the buret reading, and calculate the volume (A), if any, of 1 N hydrochloric acid consumed by the sample. Continue the titration with 1 N sodium hydroxide until the inflection point occurring at about pH 8.8 is reached, record the burette reading, and calculate the volume (B) of 1 N sodium hydroxide required in the titration between the two inflection points (pH 4 and pH 8.8). Each ml of the volume (B) - (A) of 1 N sodium hydroxide is equivalent to 136.1 mg of KH2PO4.

4.2.4.9 SODIUM HYDROXIDE PELLETS [177,178]

**Nonproprietary Names**

- **BP:** Sodium Hydroxide
- **JP:** Sodium Hydroxide
- **PhEur:** Sodium Hydroxide
- **USP-NF:** Sodium Hydroxide

**Synonyms**

Caustic soda; E524; lye; natrii hydroxidum; soda lye; sodium hydrate.

**Chemical Name**

Sodium hydroxide

**Empirical Formula and Molecular Weight**

NaOH, 40.00

**Structure**

![Structure of Sodium Hydroxide](image)

*Figure 33: Structure of Sodium Hydroxide*
Description
Sodium hydroxide occurs as a white or nearly white fused mass. It is available in small pellets, flakes, sticks, and other shapes or forms. It is hard and brittle and shows a crystalline fracture. Sodium hydroxide is very deliquescent and on exposure to air it rapidly absorbs carbon dioxide and water.

Typical Properties

Acidity/alkalinity
pH ~ 12 (0.05% w/w aqueous solution);
pH ~ 13 (0.5% w/w aqueous solution);
pH ~ 14 (5% w/w aqueous solution).

Melting point 318°C

Solubility: Practically insoluble in Ether, soluble in glycerin and ethanol

Safety
Sodium hydroxide is widely used in the pharmaceutical and food industries and is generally regarded as a nontoxic material at low concentrations. At high concentrations it is a corrosive irritant to the skin, eyes, and mucous membranes.

LD$_{50}$ (mouse, IP): 0.04 g/kg
LD$_{50}$ (rabbit, oral): 0.5 g/kg

Stability and Storage Conditions
Sodium hydroxide should be stored in an airtight nonmetallic container in a cool, dry place. When exposed to air, sodium hydroxide rapidly absorbs moisture and liquefies, but subsequently becomes solid again owing to absorption of carbon dioxide and formation of sodium carbonate.

4.2.4.10 CONCENTRATED HYDROCHLORIC ACID [179,180]

Nonproprietary Names
BP: Hydrochloric Acid
JP: Hydrochloric Acid
PhEur: Hydrochloric Acid, Concentrated
USP-NF: Hydrochloric Acid

Synonyms
Acidum hydrochloridum concentratum; chlorohydric acid; concentrated hydrochloric acid; E507.
Chemical Name
Hydrochloric acid

Empirical Formula and Molecular Weight
HCl, 36.46

Structure

Figure 34: Structure of HCl

Description
Hydrochloric acid occurs as a clear, colorless, fuming aqueous solution of hydrogen chloride, with a pungent odor. The JP XV specifies that hydrochloric acid contains 35.0–38.0% w/w of HCl; the PhEur 6.0 specifies that hydrochloric acid contains 35.0–39.0% w/w of HCl.

Typical Properties
Acidity/alkalinity pH = 0.1 (10% v/v aqueous solution)
Boiling point 110°C (constant boiling mixture of 20.24% w/w HCl)
Density 1.18 g/cm³ at 20°C
Freezing point 24°C
Solubility Miscible with water; soluble in diethyl ether, ethanol (95%), and methanol.

Method of Manufacture
Hydrochloric acid is an aqueous solution of hydrogen chloride gas produced by a number of methods including: the reaction of sodium chloride and sulfuric acid; the constituent elements; as a by-product from the electrolysis of sodium hydroxide; and as a by-product during the chlorination of hydrocarbons.

Safety
When used diluted, at low concentration, hydrochloric acid is not usually associated with any adverse effects. However, the concentrated solution is corrosive and can cause severe damage on contact with the eyes and skin, or if ingested.
LD₅₀ (mouse, IP): 1.4 g/kg(1)
LD₅₀ (rabbit, oral): 0.9 g/kg
Stability and Storage Conditions: Hydrochloric acid should be stored in a well-closed, glass or other inert container at a temperature below 30°C.

4.2.5 Formulation of Intranasal Microsphere Of Ropinirole Hydrochloride

4.2.5.1 Trial and error method:
(Preliminary experiments) previously many trials were run for the preparation of Microbeads of Ropinirole Hydrochloride by Emulsion Solvent Evaporation technique using different polymers. Trials were made by changing the temperature, stirring speeds, concentration of the polymer, Tween-80, Span-80. After so many trials, it was concluded that temperature play a very critical role in the formation of Microbeads, it is a continuous process of stirring, with the combination of hydrophilic and lipophilic surfactants. Every step in the process was optimized by performing experiments through trial and error method.

4.2.5.2 Preparation of Ropinirole Hcl Microspheres\textsuperscript{[181,182]}
Ropinirole Hcl Microspheres are prepared by Emulsion Solvent Evaporation technique using three polymers i.e., Chitosan, Carbopol 974P and Guar Gum individually and in combinations. The following are the steps for the preparation of micro beads.

- Polymer (Chitosan, Carbopol 974P and Guar Gum) was allowed to hydrate in 40ml of water for some time to achieve a viscous solution.
- Weighed quantity of Ropinirole Hcl was dispersed in 10ml of water and this was added to the polymer dispersion.
- To the above drug-gum dispersion 0.1% Tween 80 was added under constant stirring.
- Separately 200 ml of light liquid paraffin was measured in a glass beaker and to this 0.1% Span 80 was added to it.
- The oil phase was placed on a hot plate fitted with a remi stirrer.
- The aqueous phase/ polymer dispersion was added to the oil phase in a thin stream over 2 to 3 minutes.
- This emulsion was stirred at 2000 rpm and heated to 80°C for 3 to 4 hrs. The aqueous phase evaporates leaving the Microspheres dispersed in oil phase.
- The Microspheres were harvested by decantation from the oil phase and washed 3 to 4 times with 100ml aliquots of n-Hexane to make the Microbeads free from oil.
Then these are allowed to dry at 40°C for 1 hour in a hot air oven.

The micro beads were stored in tight containers till taken for further evaluation.

Table 10: Composition of Ropinirole HCL Microspheres

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<tr>
<th>FORMULATION CODE</th>
<th>DRUG (mg)</th>
<th>Chitosan % (W/V)</th>
<th>Carbopol 974P % (W/V)</th>
<th>GUAR GUM % (W/V)</th>
<th>TWEEN 80 %</th>
<th>SPAN 80 %</th>
<th>LIQUID PARAFFIN (ml)</th>
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