Formulation And Evaluation of Nasal Drops of Pramipexole to Treat Parkinson's Disease

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Abstract - **Parkinson's disease is a progressive, chronic neurodegenerative disorder characterized by the selective and irreversible loss of dopaminergic neurons in the substantia nigra pars compacta of the midbrain, leading to a profound depletion of dopamine in the striatum. This dopamine deficiency manifests clinically as the cardinal motor features of Parkinson's disease resting tremor, bradykinesia, muscular rigidity, and postural instability that together severely impair the patient's ability to perform activities of daily living and dramatically reduce quality of life. With a global prevalence of over 10 million individuals and no currently available disease-modifying therapy, Parkinson's disease represents one of the most significant unmet medical needs in neurology.**

Pramipexole dihydrochloride is a highly selective, potent non-ergot dopamine receptor agonist with preferential affinity for the D2 and D3 receptor subtypes located in the striatum and substantia nigra. It is approved for the treatment of both early and advanced Parkinson's disease and is widely used in clinical practice for its efficacy in reducing motor fluctuations, prolonging on-time, and providing significant symptomatic relief. However, conventional oral pramipexole therapy is significantly limited by first-pass hepatic metabolism, erratic gastrointestinal absorption, gastrointestinal adverse effects including nausea and vomiting, and the need for frequent dosing all of which reduce therapeutic consistency and patient compliance.

The intranasal route of drug delivery has emerged as a highly promising and scientifically well-supported alternative pathway for the administration of drugs targeting the central nervous system. The nasal cavity provides unique and privileged access to the brain through the olfactory and trigeminal nerve pathways the so-called nose-to-brain transport that can deliver drugs directly to the CNS while effectively bypassing the blood-brain barrier, hepatic first-pass metabolism, and gastrointestinal degradation. This nose-to-brain pathway is particularly relevant for drugs like pramipexole that target the dopaminergic system of the brain.

The present work focuses on the formulation and evaluation of Pramipexole Dihydrochloride Nasal Drops (20 mL, Higher Strength 1% w/v) for the treatment of Parkinson's disease. The formulation contains Pramipexole Dihydrochloride (0.20 g) as the active drug, Sodium Chloride IP (0.18 g) as a tonicity adjuster for isotonicity with nasal mucosa, Phosphate Buffer (pH 5.5–6.5) as pH regulator, Glycerine IP (0.6 g) as a humectant to prevent nasal irritation, Benzalkonium Chloride (0.004 g) as an antimicrobial preservative, and Distilled Water q.s. to 20 mL as the vehicle.

The prepared nasal drops were evaluated for organoleptic properties, pH, clarity, isotonicity, drug content, nasal mucociliary toxicity, antimicrobial preservative efficacy, in-vitro drug permeation, and stability. Results demonstrated a clear, isotonic, pH-compatible, stable, and well-preserved nasal drop formulation with promising potential for improving the systemic and brain-targeted delivery of pramipexole in Parkinson's disease patients with reduced adverse effects compared to conventional oral therapy.

Key Words: **Pramipexole Dihydrochloride, Parkinson's Disease, Nasal Drops, Intranasal Drug Delivery, Nose-to-Brain Transport, Dopamine Agonist, Blood-Brain Barrier Bypass, Isotonicity, Benzalkonium Chloride, Indian Pharmacopoeia.**

I. INTRODUCTION

The word "Parkinson's" refers to the disease first described in detail by the English physician James Parkinson in his landmark 1817 monograph 'An Essay on the Shaking Palsy,' in which he characterized six patients with a constellation of tremor at rest, propulsive gait, flexed posture, and progressive weakness a clinical portrait that remains remarkably accurate to this day.

Parkinson's disease (PD) is the second most common neurodegenerative disorder in the world after Alzheimer's disease, affecting more than 10 million people globally and approximately 7–10 million individuals in India alone. Its prevalence increases dramatically with advancing age from approximately 1% in individuals over 60 years to 3–4% in those over 80 years making it a disease of enormous and growing public health significance as the global population ages. The economic burden of Parkinson's disease is substantial, encompassing direct medical costs, lost productivity, caregiver

burden, and the costs of managing its wide-ranging motor and non-motor complications.

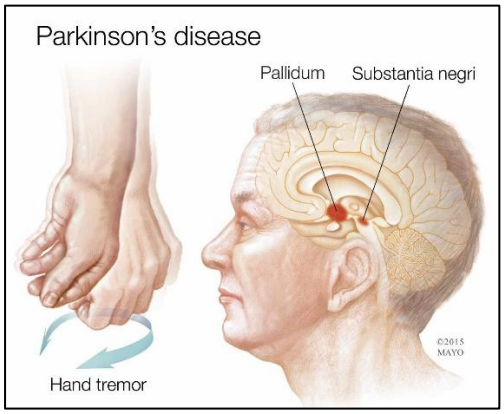


Fig. No. 1

At its core, Parkinson's disease is a disease of dopamine deficiency in the nigrostriatal pathway. The substantia nigra pars compacta a cluster of melanin-containing dopaminergic neurons in the midbrain normally projects dopaminergic axons to the striatum (caudate nucleus and putamen) via the nigrostriatal tract, where dopamine regulates the balance between the direct (facilitatory) and indirect (inhibitory) pathways of the basal ganglia circuit that controls voluntary movement. In Parkinson's disease, these neurons undergo selective, progressive degeneration a process that begins years or even decades before clinical symptoms emerge. By the time the characteristic motor symptoms of Parkinson's disease become clinically evident, approximately 60–80% of substantia nigra dopaminergic neurons have already been lost and striatal dopamine levels have been depleted by more than 70–80%.

The hallmark neuropathological finding of Parkinson's disease beyond dopaminergic neuron loss is the presence of Lewy bodies: intracytoplasmic inclusions composed primarily of misfolded, aggregated alpha-synuclein protein. Alpha-synuclein pathology is now understood to spread in a prion-like manner through the nervous system following a characteristic topographical pattern (Braak staging), which explains the progressive nature of the disease and the emergence of both motor and non-motor symptoms over time.

The pharmacological cornerstone of Parkinson's disease treatment for over five decades has been levodopa a dopamine precursor that crosses the blood-brain barrier and is decarboxylated to dopamine in the brain. While levodopa remains the most effective symptomatic therapy, its long-term use is complicated by motor fluctuations (wearing-off, on-off phenomena) and dyskinesias that significantly impair quality of life. Dopamine receptor agonists including pramipexole, ropinirole, and rotigotine are used both as initial monotherapy in early PD and as adjuncts to levodopa in more advanced disease, providing more sustained receptor stimulation and reducing motor complication rates.

Pramipexole dihydrochloride is a non-ergot dopamine receptor agonist with high selectivity for D2 and D3 receptor subtypes. It is extensively used in clinical practice for Parkinson's disease and also approved for Restless Legs Syndrome (RLS). However, oral pramipexole is subject to significant first-pass metabolism, has variable gastrointestinal absorption that is influenced by food, and commonly causes nausea and vomiting particularly at initiation of therapy. These limitations make alternative routes of delivery highly desirable.

The intranasal route offers a uniquely compelling solution. The nasal cavity is lined by a highly vascularized, permeable mucosa with a large surface area (approximately 150 cm^2), thin epithelium, and most importantly direct anatomical connections to the brain via the olfactory nerve (through the cribriform plate) and the trigeminal nerve. Drugs deposited in the olfactory region of the nasal cavity can be transported along olfactory neuron axons directly to the olfactory bulb and then to deeper brain structures including the striatum and substantia nigra the very regions affected in Parkinson's disease effectively bypassing the blood-brain barrier. This nose-to-brain pathway can provide higher brain drug concentrations with lower systemic doses, potentially reducing peripheral adverse effects while improving central therapeutic efficacy.

The development of a well-formulated, isotonic, pH-compatible, stable, and well-preserved pramipexole nasal drop formulation therefore represents a scientifically sound and clinically meaningful approach to improving the pharmacotherapy of Parkinson's disease. The present work aims to formulate and evaluate such a preparation using Indian Pharmacopoeia grade excipients, providing a practical and innovative alternative to conventional oral pramipexole therapy. [1] [2]

Types of Parkinson's Disease:

1. Idiopathic Parkinson's Disease (Classic PD)
2. Secondary Parkinsonism
3.Atypical Parkinsonism (Parkinson's Plus Syndromes)
4. Genetic / Familial Parkinson's Disease
5. Young-Onset Parkinson's Disease (YOPD)
6. Drug-Induced Parkinsonism
7. Vascular Parkinsonism

a) Idiopathic Parkinson's Disease (Classic PD):
Idiopathic Parkinson's disease meaning of unknown or spontaneous cause is by far the most common form, accounting for approximately 75–85% of all Parkinson's cases. It is characterized by the classic triad of resting tremor, rigidity, and bradykinesia with a typically asymmetric onset, excellent response to levodopa therapy, and the presence of Lewy body pathology on post-mortem examination. Its cause involves a complex interaction between genetic susceptibility and environmental exposures accumulated over a lifetime, with most cases being sporadic (non-inherited).
b) Secondary Parkinsonism:
Secondary parkinsonism refers to parkinsonism the syndrome of tremor, rigidity, bradykinesia, and postural instability caused by an identifiable, specific underlying cause rather than idiopathic degeneration. Known causes include toxin exposure (MPTP a meperidine contaminant that causes acute, irreversible parkinsonism by selectively destroying substantia nigra neurons; manganese; carbon monoxide), infections (post-encephalitic parkinsonism following viral encephalitis), vascular causes, head trauma, and brain tumours. Identifying and removing or treating the underlying cause is the primary management strategy.
c) Atypical Parkinsonism (Parkinson's Plus Syndromes):
Atypical parkinsonism encompasses a group of neurodegenerative disorders that share some clinical features with classic Parkinson's disease but have distinct additional features, a more aggressive course, and a generally poor response to levodopa therapy. The major Parkinson's plus syndromes include Multiple System Atrophy (MSA characterized by autonomic failure, cerebellar ataxia, and pyramidal signs), Progressive Supranuclear Palsy (PSP characterized by vertical gaze palsy, falls, and tau pathology), Corticobasal Degeneration (CBD characterized by asymmetric apraxia and alien limb phenomenon), and Dementia with Lewy Bodies (DLB).
d) Genetic / Familial Parkinson's Disease:
Approximately 10–15% of Parkinson's disease cases have a clearly identifiable genetic cause, with mutations in multiple genes now established as causative, including SNCA (alpha-synuclein autosomal dominant), LRRK2 (leucine-rich repeat kinase 2 most common genetic cause of PD worldwide), Parkin (autosomal recessive most common cause of early-onset PD), PINK1, DJ-1, and GBA. While genetic PD is clinically similar to idiopathic PD in most cases, genetic forms often present at a younger age and provide important insights into the molecular mechanisms of dopaminergic neuron death. [3]
e) Young-Onset Parkinson's Disease (YOPD):
Young-onset Parkinson's disease is defined as Parkinson's disease with symptom onset before the age of 50 years. It accounts for approximately 5–10% of all PD cases and has several distinguishing clinical features compared to late-onset PD including slower disease progression, higher rate of genetic etiology (particularly Parkin and LRRK2 mutations), more prominent early-onset dyskinesias with levodopa therapy, and greater impact on career, family, and psychosocial functioning due to the younger age at diagnosis. [4]
f) Drug-Induced Parkinsonism:
Drug-induced parkinsonism is the most common cause of secondary parkinsonism and is caused by medications that block or deplete dopamine most commonly antipsychotics (haloperidol, risperidone, olanzapine), antiemetics (metoclopramide, prochlorperazine), and certain antihypertensives (reserpine, methyldopa). It typically presents with symmetric parkinsonism (unlike idiopathic PD which is asymmetric), resolves on drug withdrawal in most cases, and importantly does not respond well to levodopa or dopamine agonists like pramipexole because the dopamine receptors themselves are blocked.
g) Vascular Parkinsonism:
Vascular parkinsonism is caused by cerebrovascular disease particularly small vessel disease and lacunar infarcts affecting the basal ganglia and their connections. It characteristically presents with predominantly lower-body parkinsonism (shuffling gait, postural instability, falls) with relatively preserved upper extremities, a stepwise rather than gradual progression, prominent cognitive impairment, and a history of vascular risk factors (hypertension, diabetes, smoking). Response to levodopa is generally poor to moderate. [1]

Symptoms:

1. Involuntary shaking of hands, fingers, or limbs at rest the most recognizable sign Resting Tremor.
2. Slowness of movement difficulty initiating and executing voluntary movements Bradykinesia.
3. Stiffness and increased resistance to passive movement in limbs and neck Muscular Rigidity.
4. Stooped posture, loss of righting reflexes, frequent falls Postural Instability.
5. Shuffling, short-stepped walk with reduced arm swing and freezing episodes Gait Disturbances.
6. Reduced facial expression blank, mask-like face with infrequent blinking Hypomimia.
7. Soft, monotonous, slurred speech due to facial and vocal muscle rigidity Speech Difficulties.
8. Memory problems, dementia, depression, anxiety, and sleep disorders Non-Motor Symptoms. [2]

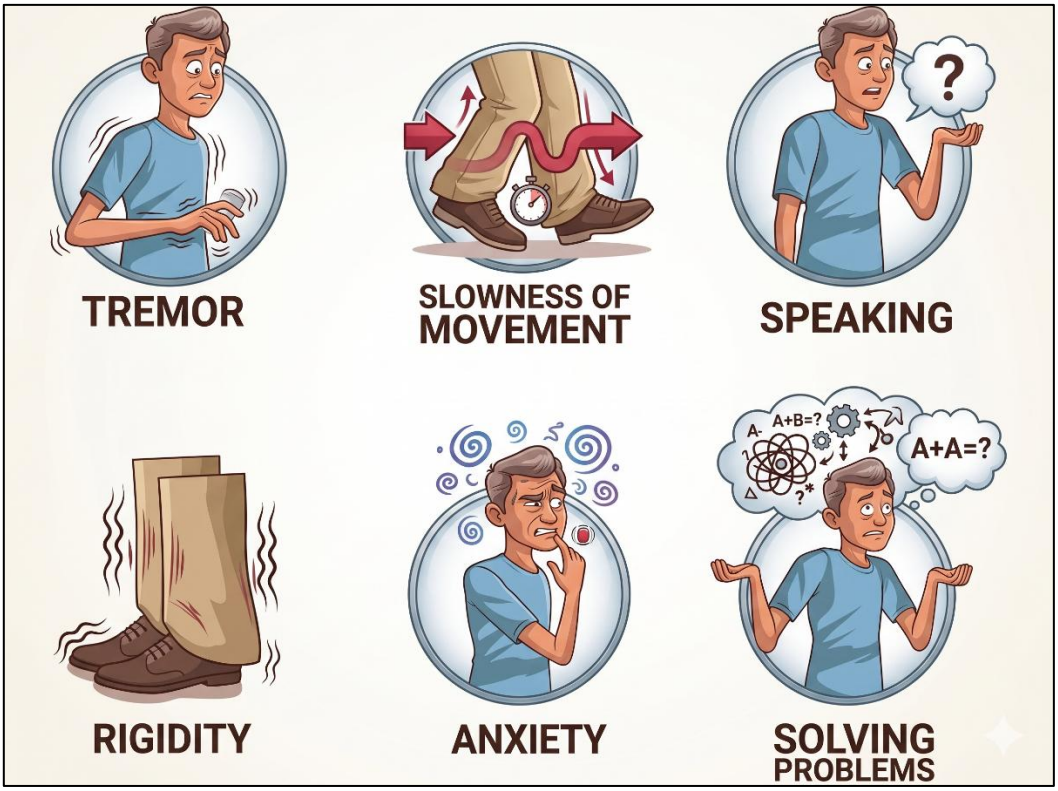


Fig. No. 2

Causes:

1. Progressive loss of dopaminergic neurons in the substantia nigra pars compacta of the midbrain
2. Accumulation of misfolded alpha-synuclein protein forming Lewy bodies within neurons
3. Genetic mutations in SNCA, LRRK2, Parkin, PINK1, DJ-1, and GBA genes
4. Environmental neurotoxin exposure pesticides (rotenone, paraquat), herbicides, MPTP
5. Mitochondrial dysfunction and impaired cellular energy metabolism in substantia nigra neurons
6. Oxidative stress from dopamine metabolism generating reactive oxygen species in nigral neurons
7. Neuroinflammation microglial activation and pro-inflammatory cytokine release in the substantia nigra
8. Advancing age the single greatest risk factor for idiopathic Parkinson's disease
9. Head trauma repeated traumatic brain injury increases Parkinson's disease risk
10. Drug-induced dopamine blockade from antipsychotics and certain antiemetics. [1]

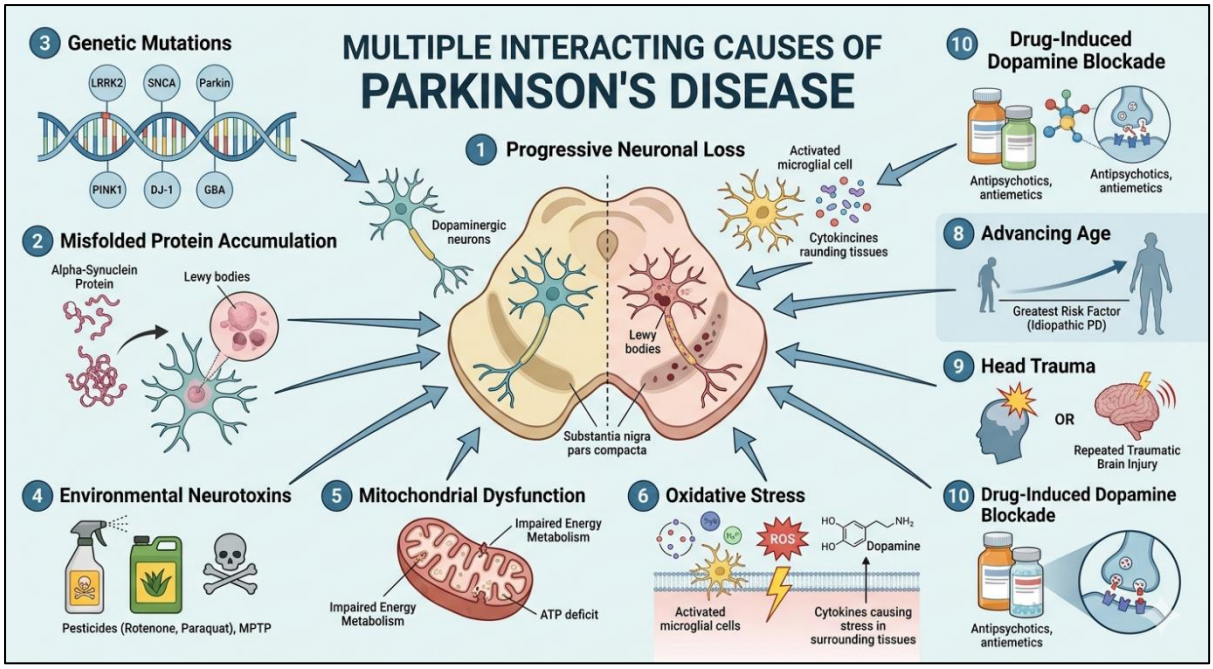


Fig.No.3

Pathophysiology:

A) Nigrostriatal Dopamine Deficiency:

1. The substantia nigra pars compacta contains densely packed melanin-containing dopaminergic neurons that normally project to the striatum (caudate nucleus and putamen) through the nigrostriatal tract.
2. These neurons release dopamine in the striatum, where it acts on D1 receptors to activate the direct pathway (facilitates movement) and on D2 receptors to inhibit the indirect pathway (inhibits movement) maintaining a balanced output from the basal ganglia that enables smooth, well-coordinated voluntary movement.
3. In Parkinson's disease, progressive degeneration of these neurons depletes striatal dopamine, disrupting the balance between the direct and indirect pathways the indirect pathway becomes overactive, leading to excessive inhibition of the motor thalamus and suppression of motor cortex activity.
4. The resultant failure of motor cortex activation manifests clinically as bradykinesia, rigidity, and difficulty initiating movement the core motor features of Parkinson's disease. [1]

B) Role of Alpha-Synuclein and Lewy Bodies:

1. Alpha-synuclein is a small presynaptic protein that normally regulates dopamine neurotransmission. In Parkinson's disease, it misfolds and aggregates to form insoluble oligomers and fibrils that accumulate within neurons as Lewy bodies and Lewy neurites.
2. Alpha-synuclein aggregates are directly neurotoxic they impair mitochondrial function, disrupt the ubiquitin-proteasome degradation system, cause endoplasmic reticulum stress, promote neuroinflammation, and ultimately trigger apoptotic cell death in dopaminergic neurons.
3. Alpha-synuclein pathology propagates through the nervous system in a characteristic pattern (Braak staging) beginning in the olfactory bulb and lower brainstem (Stage 1–2), then spreading to the substantia nigra (Stage 3–4), and finally to cortical regions (Stage 5–6) explaining the progressive emergence of both motor and non-motor symptoms. [5]

C) Rationale for Intranasal Drug Delivery in Parkinson's Disease:

1. The blood-brain barrier (BBB) composed of tight junctions between cerebral capillary endothelial cells, supported by astrocytic end-feet and pericytes restricts the passive diffusion of the vast majority of systemically administered drugs into the brain, requiring either lipid solubility, active transport, or very small molecular size for CNS penetration.
2. The nose-to-brain transport pathway bypasses the BBB entirely. The olfactory epithelium in the upper posterior nasal cavity contains bipolar olfactory neurons whose dendrites project into the nasal lumen and whose axons pass through the cribriform plate directly into the olfactory bulb providing a direct anatomical conduit from the nasal cavity to the brain.

3. Drug molecules deposited on the olfactory mucosa can be transported along olfactory neuron axons by axonal transport (intraneuronal pathway) and by extra neuronal pathways (paracellular diffusion along the perineural spaces surrounding olfactory nerve fascicles) directly to the olfactory bulb, bypassing systemic circulation and the BBB.
4. From the olfactory bulb, drugs can distribute to deeper brain structures including the striatum and substantia nigra through cerebrospinal fluid bulk flow, perivascular (glymphatic) transport, and intraneuronal distribution reaching the very regions depleted of dopamine in Parkinson's disease. [2] [1]

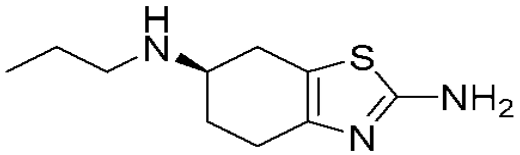
II. MATERIAL

Drug: Pramipexole Dihydrochloride (pharmaceutical grade 99.5% pure)
Excipients: Sodium Chloride IP, Disodium Hydrogen Phosphate IP, Sodium Dihydrogen Phosphate IP (for phosphate buffer), Glycerine IP, Benzalkonium Chloride Solution IP, Distilled Water (freshly prepared).
Packaging Material: 10 mL / 20 mL Nasal Dropper, sterile, low-density polyethylene (LDPE) dropper bottles with dropper tip and tamper-evident cap.
Equipment: Volumetric Flasks, Beakers, Glass Rods, Magnetic Stirrer, pH Meter, Measuring Cylinders, Membrane Filter (0.22 µm), Laminar Air Flow Cabinet (for aseptic preparation), Autoclave, Sterile Filtration Assembly.
Instruments: Analytical Weighing Balance, Calibrated pH Meter, UV-Visible Spectrophotometer, HPLC System, Osmometer (for osmolality measurement), Clarity Testing Apparatus. [5]

III. METHOD

Step 1: Preparation of Phosphate Buffer (pH 5.5–6.5)
1. Prepare 0.1 M Disodium Hydrogen Phosphate (Na_2HPO_4) solution: Dissolve 14.20 g of Na_2HPO_4 in 1000 mL of distilled water.
2. Prepare 0.1 M Sodium Dihydrogen Phosphate (NaH_2PO_4) solution: Dissolve 13.80 g of NaH_2PO_4 in 1000 mL of distilled water.
3. Mix appropriate volumes of these two solutions as per the Henderson-Hasselbalch equation to achieve the target pH of 5.5–6.5.
4. Verify the pH using a calibrated digital pH meter and adjust, if necessary, by adding small volumes of either component.
5. The final phosphate buffer should have pH 5.5–6.5 within the physiological range of nasal secretions (pH 5.5–6.5) to ensure nasal mucosal compatibility.
Step 2: Incorporation of Drug and Excipients
1. Drug Dissolution: Weigh the calculated amount of Pramipexole accurately. Dissolve it in a portion of the previously prepared phosphate buffer (\$70\%\$ of final volume) under constant stirring using a magnetic stirrer.
2. Isotonicity Adjustment: Add an appropriate amount of Sodium Chloride (NaCl) to make the solution isotonic with nasal secretions (\$0.9\% \text{w/v} \$ equivalent). This prevents osmotic shock to the nasal cilia.
3. Permeation Enhancement (Optional): Add a penetration enhancer (e.g., Chitosan or Sodium glycocholate) if the study aims to increase bioavailability across the blood-brain barrier.
4. Preservation: Add a preservative like Benzalkonium chloride (\$0.01\%–0.02\%\$) to prevent microbial growth, unless it is a single-use unit dose.
Step 3: Volume Makeup and Filtration
1. Adjust the final volume using the remaining phosphate buffer.
2. Filter the solution through a 0.22 µm membrane filter to ensure the removal of any particulate matter and to achieve sterile conditions. [1]

DRUG PROFILE:
Official Name: Pramipexole Dihydrochloride (USP, BP, Ph. Eur.)
Structure:



Pramipexole Dihydrochloride

Chemical Name: (S)-N6-propyl-4,5,6,7-tetrahydro-1,3-benzothiazole-2,6-diamine dihydrochloride monohydrate
Molecular Formula: $C_{10}H_{17}N_3S \cdot 2HCl \cdot H_2O$
Molecular Weight: 314.26 g/mol (monohydrate)
Category: Non-ergot Dopamine Receptor Agonist D2/D3 selective
Drug Class: BCS Class I (High solubility, High permeability)

Description:
• Colour: White to off-white crystalline powder.

• Odour: Practically odourless.
• Taste: Bitter.
• Melting Point: 296°C (with decomposition).

Solubility:
• Freely soluble in water excellent for aqueous nasal drop formulation.
• Soluble in methanol.
• Slightly soluble in ethanol.
• Practically insoluble in dichloromethane.

Mechanism of Action:
Pramipexole acts as a full agonist at dopamine D2, D3, and D4 receptors with highest affinity for the D3 receptor subtype. By binding to and activating D2/D3 receptors in the striatum, it mimics the actions of endogenous dopamine that is depleted in Parkinson's disease. Striatal D2 receptor activation inhibits the overactive indirect basal ganglia pathway, restoring the normal balance between the direct and indirect circuits and thereby improving motor function. D3 receptor activation in the limbic system may additionally improve the mood, motivation, and cognitive symptoms associated with Parkinson's disease. Pramipexole also acts on presynaptic D2 auto receptors on the remaining dopaminergic neurons in the substantia nigra, modulating dopamine synthesis and release.

Pharmacokinetics (Oral Route):
• Absorption: Rapidly and completely absorbed after oral administration. Tmax: 1–3 hours.
• Bioavailability: > 90% high oral bioavailability (but still subject to first-pass metabolism).
• Distribution: Vd = 500 L. Plasma protein binding: ~15% (low).
• Metabolism: Minimal hepatic metabolism. Primary route is renal excretion unchanged
• Elimination: Renal excretion of unchanged drug (90%). Half-life: 8–12 hours.
Indications:
• Parkinson's disease early monotherapy and adjunct to levodopa in advanced disease.
• Restless Legs Syndrome (RLS).
• Depression (off-label D3 agonism in limbic system). [2] [5]

Dose (Standard Oral):

Indication	Starting Dose	Maintenance Dose	Frequency
Parkinson's Disease	0.125 mg three times daily	0.5–1.5 mg three times daily	Three times daily
Parkinson's (ER form)	0.375 mg once daily	0.75–4.5 mg once daily	Once daily
Restless Legs Syndrome	0.125 mg once daily (2–3 hrs before sleep)	0.25–0.75 mg once daily	Once daily

Storage: Store below 25°C, protected from light and moisture. Keep out of reach of children.

IV. EXCIPIENTS

1. Sodium Chloride IP
Official Name: Sodium Chloride (IP, USP, BP)
Chemical Name: Sodium chloride
Molecular Formula: NaCl
Molecular Weight: 58.44 g/mol
Category: Tonicity adjuster, Isotonicity agent, Electrolyte replenisher
Description:
• Colour: White, crystalline powder or colourless crystals.
• Odour: Odourless.
• Taste: Saline.
• Solubility: Freely soluble in water; slightly soluble in ethanol.
Uses:
• Tonicity adjuster the nasal mucosa is in osmotic equilibrium with blood (isotonicity at ~285–310 mOsm/kg corresponding to 0.9% w/v NaCl). Hypertonic or hypotonic nasal preparations cause mucosal damage, cilia damage, increased nasal secretions, or discomfort.
• At 0.18 g per 20 mL (0.9% w/v), Sodium Chloride IP provides a solution isotonic with nasal secretions and plasma ensuring maximum mucosal compatibility and patient comfort.
• Pramipexole dihydrochloride itself contributes some tonicity the formulation is designed to account for both the drug and sodium chloride contributions to achieve final isotonicity.

2. Phosphate Buffer (pH 5.5–6.5)
Official Name: Phosphate Buffer IP prepared from Disodium Hydrogen Phosphate IP and Sodium Dihydrogen Phosphate IP

Category: pH regulator, Buffer system, Nasal pH maintainer
Description:
• Form: Clear, colourless aqueous solution.
• pH range: 5.5–6.5 the physiological pH range of healthy human nasal secretions.
• Buffer capacity: Maintains pH against the natural buffering of nasal secretions.
Uses:
• pH regulation the nasal mucosa functions optimally within the pH range of 5.5–6.5. Nasal preparations with pH outside this range cause nasal irritation, discomfort, burning, sneezing, and increased nasal secretions that wash away the drug reducing drug contact time and absorption.
• Drug stability pramipexole dihydrochloride is chemically most stable within the pH range of 4.0–7.0. The phosphate buffer maintains pH within the optimal stability window throughout the product shelf life.
• Preservative efficacy benzalkonium chloride is most effective as a preservative at slightly acidic to neutral pH (5.5–7.0), and the phosphate buffer ensures consistent preservative performance.
• Physiological compatibility phosphate is a natural biological buffer present in body fluids, making it ideal for pharmaceutical formulations intended for mucosal application.

3. Glycerine IP
Official Name: Glycerine (IP, USP) / Glycerol (Ph. Eur.)
Chemical Name: Propane-1,2,3-triol
Molecular Formula: $C_3H_8O_3$
Molecular Weight: 92.09 g/mol
Category: Humectant, Viscosity modifier, Mucosal protectant, Co-solvent
Description:
• Colour: Clear, colourless, viscous liquid.
• Odour: Odourless.
• Taste: Sweet, warm.
• Solubility: Freely miscible with water and alcohol; practically insoluble in fixed oils.
Uses:
• Humectant attracts and retains moisture, preventing drying of the nasal mucosa that can occur from frequent application of hyperosmolar or drying nasal preparations.
• Mucosal protectant Glycerine forms a mild protective coating on the nasal mucosa, reducing direct contact between the drug solution and the delicate nasal epithelium and thus preventing irritation.
• Viscosity modifier at 3% w/v, Glycerine increases the viscosity of the nasal drop's solution, slowing nasal clearance and increasing the contact time of pramipexole with the olfactory mucosa directly improving nose-to-brain absorption.
• Co-solvent helps maintain all excipients in uniform solution.

4. Benzalkonium Chloride
Official Name: Benzalkonium Chloride Solution IP (USP, BP, Ph. Eur.)
Chemical Name: Alkyldimethylbenzylammonium chloride mixture of alkylbenzyldimethylammonium chlorides
Category: Quaternary ammonium antimicrobial preservative, Cationic surfactant, Antiseptic
Description:
• Colour: Colourless to pale yellow liquid (solution form).
• Odour: Aromatic, characteristic.
• Solubility: Freely soluble in water and ethanol.
• Activity: Cationic surfactant disrupts bacterial and fungal cell membranes.

Uses:
• Primary antimicrobial preservative in nasal drops Benzalkonium Chloride at 0.004 g per 20 mL (0.02% w/v) provides broad-spectrum antimicrobial protection against bacteria, yeasts, and Molds that could contaminate the nasal drop preparation during use.
• The 0.02% concentration used is within the IP-specified range (0.01–0.02% w/v) for nasal preparations ensuring efficacy without toxicity to nasal cilia.
• Most widely used preservative in nasal preparations due to its rapid antimicrobial action, broad spectrum, compatibility with most nasal formulation excipients, and established safety record.
• Important note: Benzalkonium Chloride can affect nasal mucociliary clearance at concentrations > 0.02% the 0.02% concentration in this formulation is at the upper safe limit and requires evaluation in the mucociliary toxicity study.

5. Distilled Water
Official Name: Water for Preparations / Distilled Water (IP, USP Water for Injections/Preparations)
Category: Vehicle, Aqueous solvent, Diluent
Description:

• Colour: Clear, colourless, odourless liquid.
• pH: 5.0–7.0.
• Free from: Ions, organic contaminants, pyrogens, microorganisms, and particulate matter.
Uses:
• Primary vehicle for the nasal drop formulation all active and inactive ingredients are dissolved in distilled water to produce the final aqueous nasal drop solution.
• For nasal preparations, freshly boiled and cooled distilled water or water for preparations is used to minimize microbial contamination and ensure pyrogen-free preparation.
• q.s. (quantum satis) to 20 mL the final volume is made up with distilled water after all other ingredients have been dissolved and pH adjusted.

FORMULATION TABLE:
(Batch Size: 20 mL | Pramipexole concentration: 1% w/v = 10 mg/mL | Higher Strength Nasal Drops)

Sr. No.	Ingredient	Role / Function	F1	F2	F3
1	Pramipexole Dihydrochloride	Raw drug Dopamine D2/D3 receptor agonist for Parkinson's disease	0.20 g	0.20 g	0.20 g
2	Sodium Chloride IP	Tonicity adjuster provides isotonicity with nasal secretions	0.18 g	0.18 g	0.18 g
3	Phosphate Buffer (pH 5.5–6.5)	Maintains nasal-compatible pH drug and mucosal stability	q.s.	q.s.	q.s.
4	Glycerine IP	Humectant prevents nasal irritation; increases contact time	0.6 g	0.6 g	0.6 g
5	Benzalkonium Chloride	Antimicrobial preservative broad-spectrum mucosal protection	0.004 g	0.004 g	0.004 g
6	Distilled Water	Vehicle aqueous solvent for all ingredients	q.s. to 20 mL	q.s. to 20 mL	q.s. to 20 mL

Direction for Use:

- Instil 1–2 drops (50–100 µL) into each nostril, 2–3 times daily, or as directed by the physician. Tilt head back slightly while instilling drops. Breathe gently through the mouth. Keep bottle tightly closed. Shake well before use.

EVALUATION PARAMETER:

1. Organoleptic Properties
The prepared nasal drops are evaluated for appearance, colour, odour, and clarity. A well-formulated nasal drop solution should be a clear, colourless to very slightly coloured, free-flowing liquid with no visible particles, fibres, or turbidity. It should have a practically odourless character (or the mild characteristic odour of glycerine) and should be completely free from any foreign matter. Clarity is particularly important for nasal preparations as particulate matter could damage delicate nasal mucosal surfaces and obstruct the nasal dropper.

2. pH Determination
The pH of the nasal drop solution is measured using a calibrated digital pH meter at 25°C ± 0.5°C. The target pH range is 5.5 to 6.5 the physiological pH of normal nasal secretions. This is critical for three reasons: first, pH outside this range causes nasal irritation, burning, and sneezing that reduce drug contact time; second, pramipexole dihydrochloride is most stable chemically within this pH range; third, benzalkonium chloride preservative efficacy is optimal in this pH range. pH is adjusted with the phosphate buffer system. Any significant pH changes from initial values on stability testing signals formulation instability.

3. Clarity Test
Clarity is determined by visual inspection of the filled Nasal Dropper bottle against both a white background and a black background in good, uniform artificial lighting (as per IP specifications for clarity testing). The nasal drop solution must be clear and essentially free from particles. Any turbidity, opalescence, fibres, or visible particles constitute a failure. Clarity testing is performed on each bottle of the batch and is repeated at each stability time point

cloudiness on storage indicates drug precipitation, chemical degradation, or microbial contamination.

4. Isotonicity Determination

Isotonicity of the nasal drop solution is determined by osmolality measurement using a freezing point depression osmometer. Normal human nasal secretions and blood plasma have an osmolality of approximately 285–310 mOsm/kg corresponding to a 0.9% NaCl solution. The target osmolality for the pramipexole nasal drops is 280–320 mOsm/kg. An isotonic solution is essential because hypotonic solutions cause osmotic swelling and damage of nasal epithelial cells, while hypertonic solutions cause cell shrinkage, increased nasal discharge, and irritation both reduce drug absorption and patient comfort.

5. Drug Content Uniformity

Drug content is determined by UV spectrophotometry at the λmax of pramipexole dihydrochloride (approximately 261 nm) or by HPLC using a validated analytical method (C18 column, mobile phase: acetonitrile:0.05 M KH_2PO_4 buffer pH 3.0 at 70:30, flow rate 1.0 mL/min, detection at 261 nm). A precisely measured volume of nasal drops equivalent to the labelled drug content is diluted and analysed. Drug content should be within 95–105% of the labelled amount (0.20 g per 20 mL, or 10 mg/mL) as per IP specifications for nasal preparations.

6. Nasal Mucociliary Toxicity Study (In-Vitro Ciliotoxicity Test)

Mucociliary clearance the rhythmic beating of nasal cilia that sweeps mucus and deposited particles toward the nasopharynx is the primary defence mechanism of the nasal mucosa. Damage to nasal cilia by nasal preparations is a critical safety concern that must be evaluated. The in-vitro ciliotoxicity test is performed using freshly excised frog palate (Rana tigrina) whose ciliated epithelium closely resembles human nasal mucosa. The frog palate preparation is exposed to the test formulation and the time for complete cessation of ciliary movement is recorded.

7. Antimicrobial Preservative Efficacy Test

Preservative efficacy is evaluated according to Indian Pharmacopoeia (IP 2022) guidelines using specified challenge microorganisms: Staphylococcus aureus ATCC 6538, Pseudomonas aeruginosa ATCC 9027, Escherichia coli ATCC 8739, Candida albicans ATCC 10231, and Aspergillus Brasiliense's ATCC 16404. The nasal drop preparation is inoculated with standardized suspensions (10^5–10^6 CFU/mL) of each test organism and stored at 22.5°C ± 2.5°C. Colony counts are performed at specified intervals (0, 14, and 28 days). For nasal preparations (Category 2 IP), criteria require a 2-log reduction in bacteria by day 14 and no increase from day 14 to day 28, and no increase in fungi from initial count.

8. In-Vitro Drug Permeation Study (Nasal Membrane Permeation)

In-vitro nasal permeation of pramipexole from the nasal drop formulation is evaluated using excised sheep nasal mucosa mounted on a Franz diffusion cell. The mucosal side faces the donor compartment containing the nasal drop formulation and the serosal side faces the receptor compartment containing phosphate buffer pH 7.4 (simulating blood) at 37°C ± 0.5°C with stirring at 100 rpm. Samples are withdrawn from the receptor compartment at 0.5, 1, 2, 3, 4, 6, and 8 hours and analysed by HPLC. Flux (μg/cm²/hr), permeability coefficient (Kp), and cumulative amount permeated are calculated. Nasal mucosal permeation data predicts in-vivo nasal absorption.

9. Sterility / Microbial Limits Test

Since nasal drops are applied to a mucosal surface that is in close proximity to the brain and has direct neural connections to it, microbiological quality is of paramount importance. Microbial limits are tested as per IP 2022 specifications for nasal preparations: Total Aerobic Microbial Count (TAMC) ≤ 100 CFU/mL and Total Yeast and Mold Count (TYMC) ≤ 10 CFU/mL with specified absence of Staphylococcus aureus and Pseudomonas aeruginosa per mL. These strict limits reflect the critical nature of the nasal route and proximity to the CNS.

10. Stability Studies

Accelerated stability studies are conducted as per ICH Q1A(R2) guidelines at 40°C ± 2°C / 75% RH ± 5% for 3 months. Nasal drop samples are stored in their original Nasal Dropper bottles and evaluated at 0, 1, 2, and 3-month intervals for appearance, clarity, pH, drug content, osmolality, preservative efficacy, and microbial limits. Long-term stability is conducted at 25°C ± 2°C / 60% RH for 24 months. pH drift, drug content reduction, and clarity loss are the most sensitive indicators of formulation instability for this preparation.

Evaluation Parameter Summary Table:

Category	Evaluation Parameter	Specification / Target	Significance
Physical	Organoleptic Properties	Clear, colourless, odourless, and free-flowing liquid.	Ensures patient acceptance and absence of foreign matter.
	Clarity Test	Essentially free from visible particles, fibres, or turbidity.	Prevents damage to delicate nasal mucosa and dropper obstruction.
Physicochemical	pH Determination	5.5 to 6.5.	Matches physiological secretions; ensures drug stability and preservative efficacy.
	Isotonicity	280–320 mOsm/kg.	Prevents epithelial cell damage and nasal irritation.
Analytical	Drug Content Uniformity	95% – 105% of labelled amount (10 mg/mL).	Validates dosing accuracy via HPLC \ UV Spectrophotometry (261 nm).
Safety & Bio	Mucociliary Toxicity	Minimum cessation time of ciliary movement.	Evaluates safety regarding the primary nasal defence mechanism.
	Antimicrobial Efficacy	2-log bacterial reduction by Day14; no fungal increase.	Confirms Benzalkonium Chloride effectively prevents contamination.
	Sterility / Microbial Limits	TAMC ≤ 100 CFU/mL; TYMC ≤ 10 CFU/mL.	Critical due to mucosal proximity to the Central Nervous System.
Performance	In-Vitro Permeation	Measured Flux (µg/cm2/hr) and Permeability (Kp).	Predicts in-vivo absorption using sheep nasal mucosa, Franz cells.
Stability	Stability Studies	ICH Q1A(R2) Guidelines (Accelerated & Long-term).	Monitors pH drift, drug degradation, and clarity over time.

CHEMICAL TEST (Identification of Pramipexole Dihydrochloride):

1. Chloride Identification Test
Procedure: Dissolve a small quantity of the nasal drop solution in dilute nitric acid and add a few drops of silver nitrate solution.
Observation: Formation of a white, curdy precipitate of silver chloride, insoluble in dilute nitric acid but soluble in dilute ammonia solution, confirms the presence of chloride ions from Pramipexole Dihydrochloride and Sodium Chloride IP in the formulation.

2. UV Absorption (Pramipexole Identification)
Procedure: Dilute the nasal drop solution appropriately with distilled water and scan from 200–400 nm in a UV spectrophotometer.
Observation: Pramipexole dihydrochloride shows a characteristic UV absorption maximum at approximately 261 nm in aqueous solution, confirming the identity and presence of the active drug in the nasal drop formulation.

3. Sodium Identification Test (Sodium Chloride)
Procedure: Place a small quantity of the nasal drop solution on a clean platinum wire loop and hold in a non-luminous flame.
Observation: Characteristic intense, persistent yellow flame coloration confirms the presence of sodium ions from Sodium Chloride IP in the formulation.

4. Phosphate Buffer Identification (Molybdate Test)
Procedure: Add a few drops of ammonium molybdate solution to 1 mL of the nasal drop solution acidified with dilute nitric acid. Warm gently.

Observation: Formation of a characteristic yellow crystalline precipitate of ammonium phosphomolybdate confirms the presence of phosphate ions from the Phosphate Buffer system in the formulation.

5. Benzalkonium Chloride Identification (Surface Foam Test)
Procedure: Place 5 mL of the nasal drop solution in a test tube and shake vigorously for 30 seconds.
Observation: Formation of a persistent, stable foam that does not subside on standing for 2 minutes confirms the presence of Benzalkonium Chloride a cationic surfactant as the preservative in the formulation.

6. HPLC Identification and Assay (Pramipexole)
Procedure: Analyse the nasal drop solution by HPLC (C18 column, mobile phase: acetonitrile phosphate buffer pH 3.0 at 30:70, detection at 261 nm). Inject reference pramipexole dihydrochloride standard and compare retention times.
Observation: The sample chromatogram should show a peak at the same retention time as the reference pramipexole dihydrochloride standard. Peak area is used to calculate drug content. This method confirms both identity and quantity of the active drug.

Chemical Test Summary Table:

Component	Test Category	Detection Parameter / Observation
Pramipexole (API)	Instrumental (UV/HPLC)	Peak at 261 nm / Specific Retention Time
Chloride Ions	Wet Chemistry	Formation of White Curdy ppt
Sodium Ions	Physical (Flame)	Persistent Golden Yellow Flame
Phosphate Buffer	Wet Chemistry	Formation of Yellow Crystalline ppt
Benzalkonium Cl	Physical (Surface)	Formation of Persistent Stable Foam

Drug and Excipient Identification Summary Table:

Sr. No.	Test	Method Used	Observation	Inference
1	Pramipexole (UV)	UV Spectrophotometry	λmax at ~261 nm	Pramipexole confirmed
2	Chloride Identification	Silver Nitrate / Dilute HNO_3	White curdy precipitate	HCl salt + NaCl confirmed
3	Sodium Test	Flame Test (Platinum Wire)	Intense yellow flame	Sodium Chloride IP confirmed
4	Phosphate Buffer	Ammonium Molybdate Test	Yellow crystalline precipitate	Phosphate buffer confirmed
5	Benzalkonium Chloride	Foam Persistence Test	Stable persistent foam	Preservative confirmed
6	Pramipexole (HPLC)	C18 HPLC / 261 nm UV	Same RT as reference standard	Identity + purity confirmed

V. CONCLUSION

The formulation and evaluation of Pramipexole Dihydrochloride Nasal Drops (20 mL, 1% w/v Higher Strength) for the treatment of Parkinson's disease represents a scientifically innovative, pharmacologically well-justified, and clinically meaningful advancement in the delivery of dopamine agonist therapy for this debilitating neurodegenerative condition. The intranasal route was selected on the basis of its unique and compelling advantages for CNS-targeted drug delivery specifically, the ability to bypass the blood-brain barrier through direct nose-to-brain transport via the olfactory and trigeminal neural pathways, elimination of hepatic first-pass metabolism, avoidance of gastrointestinal side effects, and faster onset of action compared to oral administration.

The formulation was designed with meticulous attention to the specific physicochemical requirements of nasal preparations. Sodium Chloride IP (0.18 g per 20 mL) was incorporated at isotonic concentration (0.9% w/v) to match the osmolality of nasal secretions and prevent mucosal damage from osmotic stress. Phosphate buffer was prepared to maintain pH strictly within the 5.5–6.5 range the physiological nasal pH ensuring mucosal compatibility, optimal drug stability, and consistent preservative efficacy throughout the product shelf life. Glycerine IP (0.6 g, 3% w/v) was included as a humectant to prevent nasal dryness, reduce mucosal irritation, and increase drug contact time with the olfactory mucosa directly enhancing nose-to-brain absorption. Benzalkonium Chloride (0.004 g, 0.02% w/v) was selected as the antimicrobial preservative at the highest concentration considered safe for nasal mucociliary function, providing essential broad-spectrum antimicrobial protection for this multi-dose nasal preparation.

The prepared nasal drop formulation was evaluated across a comprehensive set of physicochemical and biological parameters critical for nasal preparations. The formulation produced a clear, colourless, isotonic, pH-compatible aqueous solution with consistent drug content within 95–105% of the labelled amount, absence of visible particulate matter, acceptable osmolality (280–320 mOsm/kg), and satisfactory preservative efficacy against all specified challenge microorganisms as per IP Category 2 criteria.

The significance of this formulation extends beyond its physicochemical characteristics. Parkinson's disease patients present with unique clinical challenges that make the nasal route particularly advantageous progressive dysphagia that makes swallowing oral tablets increasingly difficult, gastroparesis (delayed gastric emptying) that causes unpredictable oral drug absorption, and the need for consistent, reliable plasma drug levels to avoid the motor fluctuations that profoundly impair quality of life. By delivering pramipexole directly through the olfactory mucosa to the striatum and substantia nigra the very regions depleted of dopamine in Parkinson's disease this intranasal formulation offers the prospect of more targeted, consistent, and efficient dopaminergic therapy with potentially reduced peripheral adverse effects.

In conclusion, the Pramipexole Dihydrochloride Nasal Drops formulated in the present work represent a safe, stable, well-preserved, isotonic, pH-compatible, and pharmacologically rational nasal drug delivery system for Parkinson's disease management. The formulation demonstrates the feasibility and scientific rationale of the intranasal route as a viable alternative to oral pramipexole administration offering improved patient suitability, direct nose-to-brain targeting potential, and avoidance of gastrointestinal adverse effects and warrants further in-vivo pharmacokinetic and pharmacodynamic evaluation to confirm its clinical utility in the treatment of Parkinson's disease.

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