Formulation And Evaluation of Nasal Spray for Anti-Depressant Therapy

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***Abstract*—There is mounting evidence that the pathophysiology of anxiety and depression, two of the most common mental health conditions in the world, is related to neuroinflammation. Clarifying the cellular and molecular mechanisms underlying these conditions and assessing therapeutic interventions has been made possible in large part by experimental animal models. A common psychoactive substance and nonselective adenosine receptor antagonist, caffeine has been shown to have modulatory effects on neuroinflammatory pathways. Caffeine can reduce oxidative stress, inhibit glial activation, suppress pro-inflammatory cytokines like IL-1β, TNF-α, and IL-6, and alleviate anxiety- and depressive-like behaviours, according to a systematic review of rodent studies.Similarly, it has been demonstrated that early-life stressors like maternal separation can cause long-term depressive-like behaviours linked to increased hippocampal neuroimmune responses. In these models, the nonsteroidal anti-inflammatory drug indomethacin has demonstrated antidepressant-like effects by reducing behavioural deficits and downregulating inflammatory mediators such as iNOS, TLR4, NLRP3, TNF-α, and IL-1β. All of these results support the therapeutic potential of anti-inflammatory and neuromodulatory drugs and emphasise the crucial role that neuroinflammation plays in depression. By improving brain targeting through the nose-to-brain pathway, these agents can be incorporated into novel drug delivery systems like nasal sprays, increasing efficacy and lowering systemic side effects. Therefore, by modifying neuroinflammatory mechanisms, nasal spray formulations offer a promising approach to managing depression.**

***Index Terms*—1β, TNF-α, and IL-6, IL-1β, iNOS, TLR4, NLRP3, TNF-α**

I. INTRODUCTION

In recent years, mental health challenges have increased rapidly among young people due to stress, busy lifestyles, and social pressure. Therefore, this project focuses on developing an effective nasal spray formulation to improve brain health and patient well-being. Depression: depression is an affective disorder characterised by symptoms like sad mood, loss of interest and pleasure, low energy, worthlessness, guilt, psychomotor retardation or agitation, change in appetite and/or sleep, melancholia, suicidal thoughts, etc. It may be a unipolar or a bipolar cyclic disorder in which cycles of mood swings from mania to depression occur over time. The mood change may have a psychotic basis with delusional thinking. Anxiety and depression are the leading psychiatric disorders now.

Persistent sadness, a lack of interest in activities, low energy, difficulty concentrating, and changes in sleep and appetite are all signs of depression, a serious and long-lasting mental illness. It affects people of all ages and is one of the main causes of disability in the world. In addition to lowering quality of life, if treatment is not received, the illness may cause serious side effects like suicidal thoughts and actions.

Numerous factors, including neurotransmitter imbalance (serotonin, dopamine, and norepinephrine), neuroinflammation, hormonal abnormalities, and genetic predisposition, contribute to the complex pathophysiology of depression. Oral antidepressants such as monoamine oxidase inhibitors (MAOIs), tricyclic antidepressants (TCAs), and selective serotonin reuptake inhibitors (SSRIs) are the mainstay of conventional treatment.

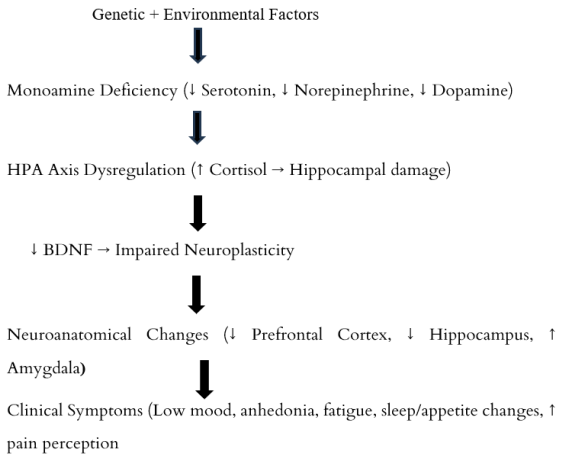


Figure 1: Pathophysiology pathways of depression.

Intra Nasal Route: Nose to Brain Drug Delivery

For strong medications with little to no oral bioavailability, the IN route is an appropriate delivery method. It is an excellent alternative to several traditional dosage forms because it is a safe, non-invasive, and appropriate form and strategy. They have the advantage of overcoming the BBB's limitations by offering a direct transport route via the nasal route to the target CNS. Delivering the appropriate drug concentrations to the site of action is the main goal of these drug delivery routes. Furthermore, it is possible to reduce both physical clearance and drug degradation through metabolism.

Rapid drug absorption to the brain is made possible by the highly permeable nasal epithelium's large surface area, porous endothelial membrane, high total blood flow, and avoidance of first-pass metabolism. Numerous therapeutic agents, including both small and large molecules, can be administered to the central nervous system via the intranasal route. Furthermore, neither the therapeutic agent's modification nor the drug's coupling to a carrier is necessary for nasal drug delivery. Nasal drug delivery, which offers strong advantages over other drug delivery techniques, has long been a major area of development for pharmaceutical and medical device companies.

- Advantages Of Nasal Drug Delivery System

1. Intranasal administration offers several practical advantages from the viewpoint of patients
1. (non-invasiveness, essentially painless, ease of drug delivery and favourable tolerability profile)
2. Rapid drug absorption.
3. Quick onset of action.
4. Hepatic first – pass metabolism is absent.
5. The bioavailability of larger drug molecules can be improved by means of an absorption
6. enhancer or other approach.
7. Better nasal bioavailability for smaller drug molecules.

- Limitation Of Nasal Drug Delivery System

1. Dose is limited because of the relatively small area available for the absorption of the drug.
2. Time available for drug absorption is limited.
3. A diseased condition of the nose impairs drug absorption.
4. The absorption enhancers used to improve the nasal drug delivery system may have
1. histological toxicity, which is not yet clearly established.
5. Absorption surface area is less when compared to the GIT.
6. Certain surfactants used as chemical enhancers may disrupt and even dissolve the membrane in high concentrations.

Nasal Anatomy and Physiology

The nasal cavity is lined with a mucus layer and hairs, which are involved in those functions,
trapping inhaled particles and pathogens. Moreover, resonance of produced sounds,
mucociliary clearance MMC, immunological activities and metabolism of endogenous
substances are also essential functions of nasal structures. The human nasal cavity has a total
volume of 15-20 mL and a total surface area of approximately 150 cm2. The nasal halves
consist of four areas (nasal vestibule, atrium, respiratory region and olfactory region) that are
distinguished according to their anatomic and histological characteristics.

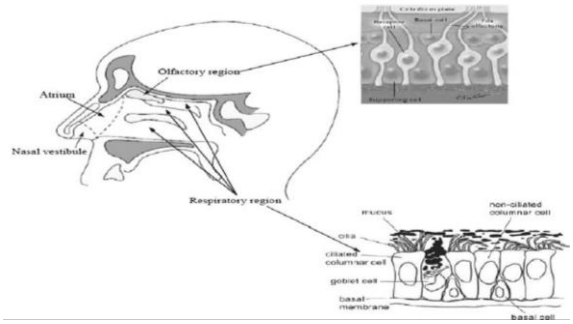


Figure 2: Anatomy and histology of the human nasal cavity.

The nasal cavity consists of three main regions are nasal vestibule, the atrium and the respiratory region.

1. Nasal vestibule:

In this area of the nasal cavity, there are nasal hairs, also called vibrissae, which filter the inhaled particles. Nasal vestibular characteristics are desirable to afford high resistance against toxic environmental substances, but, at the same time, the absorption of substances, including drugs, becomes very difficult in this region.

2. Atrium:

The atrium is the intermediate area between the nasal vestibule and the respiratory region. Its anterior section is constituted by a stratified squamous epithelium, and the posterior area by pseudo-stratified columnar cells.

3. Respiratory region:

It is divided into superior, middle and inferior turbinates, which are projected from the lateral wall. These specialised structures are responsible for humidification and temperature regulation of inhaled air. Between them, there are spaces, called meatus, which are passageways where airflow is created to ensure close contact of the inhaled air with the respiratory mucosal surface. The inferior and middle meatus receive nasolacrimal ducts and paranasal sinuses, which are air-filled pockets located inside the bones of the face and around the nasal cavity. The nasal respiratory mucosa, considered the most important section for delivering drugs systemically, is constituted by the epithelium, basement membrane and lamina propria. Nasal mucus is indispensable for several physiological functions, such as humidification and warming of the inhaled air, and also offers physical and enzymatic protection of the nasal epithelium against several foreign compounds, including drugs. The presence of mucin in the nasal mucus layer is crucial because it may trap large molecular weight drugs, such as peptides and proteins. Beneath it, there is the lamina propria, which is richly supplied with blood vessels, including many very permeable fenestrated capillaries, nerves, glands and immune cells. The last ones produce immunoglobulin and antibodies that confer immunological protection against bacteria and viruses.

Drug Delivery Pathway

Conventionally, intranasally administered drugs are absorbed by the capillary blood vessels present in the nasal mucosa and go into the bloodstream. The nasal mucosa receives blood supply from the branching of the carotid artery in the form of maxillary, ophthalmic, and facial arteries; hence, it is highly vascularized in nature. The maxillary artery provides blood supply to the respiratory mucosa, whereas the branches of the ophthalmic artery supply blood to the olfactory mucosa. The respiratory mucosa has a number of blood vessels in comparison to the olfactory mucosa; thus, the respiratory mucosa is a supreme site for systemic absorption of therapeutics. Smaller as well as larger molecules can enter the systemic circulation from the blood vessels in the respiratory region (because it constitutes a mixture of continuous and fenestrated endothelial). Drugs can also show counter-current transfer in which the drug molecules may enter the veins of the nasal passages; from there, these molecules can quickly transfer to the brain and spinal cord through the carotid artery. However, there are some problems are associated with the systemic circulation, such as drug elimination via hepatic first-pass metabolism and renal mechanisms, as well as via other factors such as enzymatic degradation, drug binding to plasma proteins, possible peripheral side effects, and limiting access through the BBB.

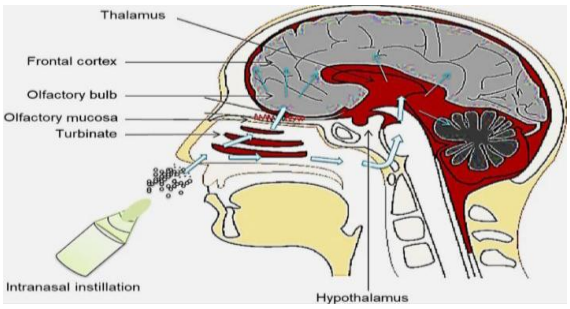
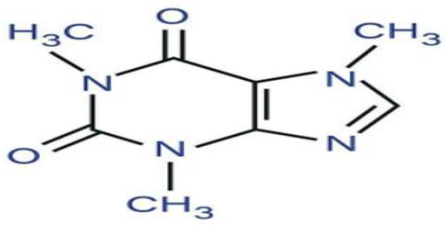


Figure 3: Route of drug transport

II. DRUG PROFILES

- CAFFEINE: Caffeine is odourless and has a characteristic bitter taste. It is a colourless powder, moderately soluble in water.
- Chemical Name: 1, 3, 7-Trimethylpurine-2,6-dione
- Molecular Formula: $C_8H_{10}N_4O_3$
- Molecular weight:194.19 g/mol



Caffeine

- Pharmacodynamics

Caffeine stimulates the central nervous system (CNS), heightening alertness and sometimes causing restlessness and agitation. It relaxes smooth muscle, stimulates the contraction of cardiac muscle, and enhances athletic performance. Caffeine promotes gastric acid secretion and increases gastrointestinal motility. It is often combined in products with analgesics and ergot alkaloids, relieving the symptoms of migraine and other types of headaches. Finally, caffeine acts as a mild diuretic.

- Pharmacokinetics:

Absorption Caffeine is rapidly absorbed after nasal administration, reaching peak plasma concentration within 30 minutes to 2 hours after administration. After nasal administration, the onset of action takes place within 45 to 1 hour. The peak plasma level for caffeine ranges from 6-10mg/L.

- Distribution

Caffeine has the ability to rapidly cross the blood-brain barrier. It is water and fat-soluble and is distributed throughout the body. Caffeine concentrations in the cerebrospinal fluid of preterm newborns are similar to the concentrations found in the plasma. The mean volume of distribution of caffeine in infants is 0.8-0.9 L/kg and 0.6 L/kg in the adult population.

- Metabolism

Caffeine metabolism occurs mainly in the liver via the cytochrome CYP1A2 enzyme. The products of caffeine metabolism include paraxanthine, theobromine, and theophylline. The first step of caffeine metabolism is demethylation, yielding paraxanthine (a major metabolite), followed by theobromine and theophylline, which are both minor metabolites. They are then excreted in urine as urates after additional metabolism.

- Mechanism of action

Caffeine exerts several actions on cells, but the clinical relevance is poorly understood. One of the probable mechanisms is the inhibition of nucleotide phosphodiesterase enzymes, adenosine receptors, regulation of calcium handling in cells, which participates in adenosine receptor antagonism.
Caffeine demonstrates antagonism of all 4 adenosine receptor subtypes (A1, A2a, A2b, A3) in the central nervous system. Caffeine's effects on alertness and combating drowsiness are specifically related to the antagonism of the A2a receptor.

- Clearance

The clearance of caffeine varies, but on average, it is about 0.078 L/kg/h (1.3 mL/min/kg).

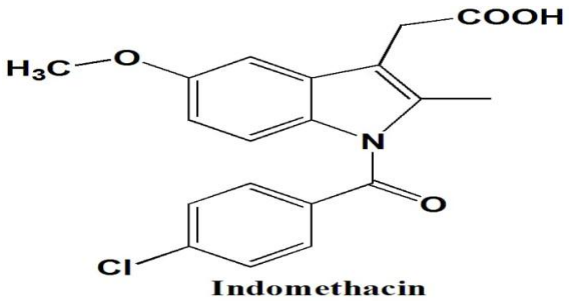
- Toxicity

In the case of caffeine overdose, seizures may occur, as caffeine is a central nervous system stimulant. It should be used with extreme caution in those with epilepsy or other seizure disorders. Symptoms of overdose may include nausea, vomiting, diarrhoea, and gastrointestinal upset.

- Indomethacin:

Indomethacin is a white to light-yellow crystalline powder. It is practically insoluble in water but soluble in organic solvents like ethanol, chloroform, and ether.

- Chemical Name: 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid
- Molecular Formula:$C_{19}H_{16}ClNO_4$
- Molecular Weight: 357.79 g/mol



Indomethacin

- Pharmacodynamics:

Indomethacin is a potent non-steroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic properties. It effectively reduces inflammation, relieves pain, and lowers fever. It is also uniquely used in neonates to help close a patent ductus arteriosus (PDA). Unlike some other NSAIDs, it is noted for its strong effects on cerebral blood flow and its efficacy in treating specific "indomethacin-responsive" headache disorders.

- Pharmacokinetics:

Absorption Indomethacin is a lipophilic drug that is readily absorbed through the nasal mucosa due to its high permeability and the rich blood supply of the nasal cavity. The study shows that intranasal administration allows rapid entry of the drug into systemic circulation while avoiding first-pass metabolism, and its absorption is comparable to oral dosing.

- Distribution

After absorption through the nasal mucosa, indomethacin enters the systemic circulation directly via the highly vascular nasal tissues. Its lipophilic nature supports efficient membrane permeation and distribution throughout the body.

- Metabolism

Intranasal delivery allows indomethacin to bypass first-pass hepatic metabolism, since the drug is absorbed directly into systemic circulation through the nasal mucosa. This means a larger fraction of the drug reaches circulation unchanged compared to oral administration.

Once absorbed, indomethacin undergoes its normal systemic metabolism in the liver, primarily through O-demethylation and N-deacylation, forming inactive metabolites. These metabolites may further undergo conjugation (mainly glucuronidation) before elimination.

- Excretion

Although not specifically discussed in the paper, indomethacin is eliminated mainly via renal and biliary routes after hepatic metabolism. The drug and its metabolites are excreted in urine (major portion) and partly in faeces. Intranasal delivery does not significantly alter the excretion pathway; it only enhances systemic availability before elimination.

III. AIM AND OBJECTIVE

Aim:

To develop and evaluate a novel intranasal drug delivery system that combines caffeine and indomethacin to provide a rapid and effective treatment for depression. This project aims to bypass the blood-brain barrier (BBB) via the nose-to-brain pathway to enhance the therapeutic concentration of these drugs in the central nervous system (CNS) while minimising systemic side effects.

Objective:

1. Increase drug absorption.
2. Improve drug bioavailability.
3. Enhance the onset of action
n time.
4. Improve patient compliance through painless and easy drug delivery.
5. Avoid hepatic first-pass metabolism.

IV. PLAN OF WORK

1.Selection of Route of Administratio

Intranasal route was selected for drug delivery due to its rapid absorption, avoidance of first-pass metabolism, and direct nose-to-brain delivery potential.

2.Selection of Drug

Caffeine and Indomethacin were selected as the active pharmaceutical ingredients (API) for formulation development.

3. Preformulation Studies

Reformulation studies of drug and excipients were carried out to evaluate compatibility and suitability for nasal spray formulation.

4.Formulation Design: The nasal spray formulation was designed using suitable excipients such as:
PEG 200 – Solubilizer
Propylene Glycol – Co-solvent
Sodium Chloride – Isotonicity enhancer
Phosphate Buffer – pH maintainer
Tween 80 – Surfactant
HPMC – Viscosity enhancer
Benzalkonium Chloride – Preservative
Sterile Water – Vehicle

5.Preparation of Nasal Spray
The formulation was prepared by dissolving the drugs and excipients in sterile water with continuous stirring to obtain a clear and homogeneous nasal spray solution.
Evaluation of Formulation
The prepared formulation was evaluated for:
Physical appearance
Ph Viscosity
Pump delivery
Sterility test
Drug content uniformity

Master Formula

Sr. no	Ingredients	Quantity	Roles
1	Caffeine	0.01g	API
2	Indomethacin	0.05g	API
3	Polyethylene Glycol 200 (PEG200)	0.5ml	Solubilizer
4	Propylene Glycol	0.02ml	Co-solvent
5	Sodium Chloride	0.09g	Isotonicity Enhancer
6	Phosphate Buffer	0.02g or q.s.	Maintains pH
7	Polysorbate 80 (Tween 80)	0.02ml	Surfactant
8	Hydroxypropyl methylcellulose (HPMC)	0.03g	Viscosity enhancer
9	Benzalkonium Chloride	0.001g	Preservative
10	Sterile Water	Q.S. 10ml	Vehicle

Table 1: Master formula and functional roles of ingredients.

2. Experimental Work
Method Of Preparation
Step1: Weigh all the ingredients accurately.

Step2: Preparation Of Buffere
Dissolve sodium chloride in sterile water.Add sodium dihydrogen phosphate and disodium hydrogen phosphate.Adjust the pH to 5.5–6 using buffer solution.

Step 3: Preparation of Drug Solution
Take PEG 400 in a clean beaker.Add caffeine and stir until completely dissolved.Add indomethacin slowly with continuous stirring.Mild heating may be used if required for complete dissolution.

Step 4: Addition of Surfactant
Add Polysorbate 80 (Tween 80) to the drug solution.Stir gently to maintain uniform solubility.

Step 5: Preparation of Polymer Solution
Disperse HPMC in a small quantity of sterile water.Stir continuously and allow hydration to obtain a uniform polymer solution.

Step 6: Mixing
Add the drug solution and polymer solution slowly into the buffer solution.Stir continuously until a clear and uniform solution is obtained.

Step 7: Addition of Preservative
Add benzalkonium chloride to the formulation.Mix gently for uniform distribution.

Step 8: Make Up the Volume
Add sterile water q.s. to make the final volume up to 10 mL.

Step 9: Filtration
Filter the prepared solution using suitable membrane filter paper to remove particulate matter

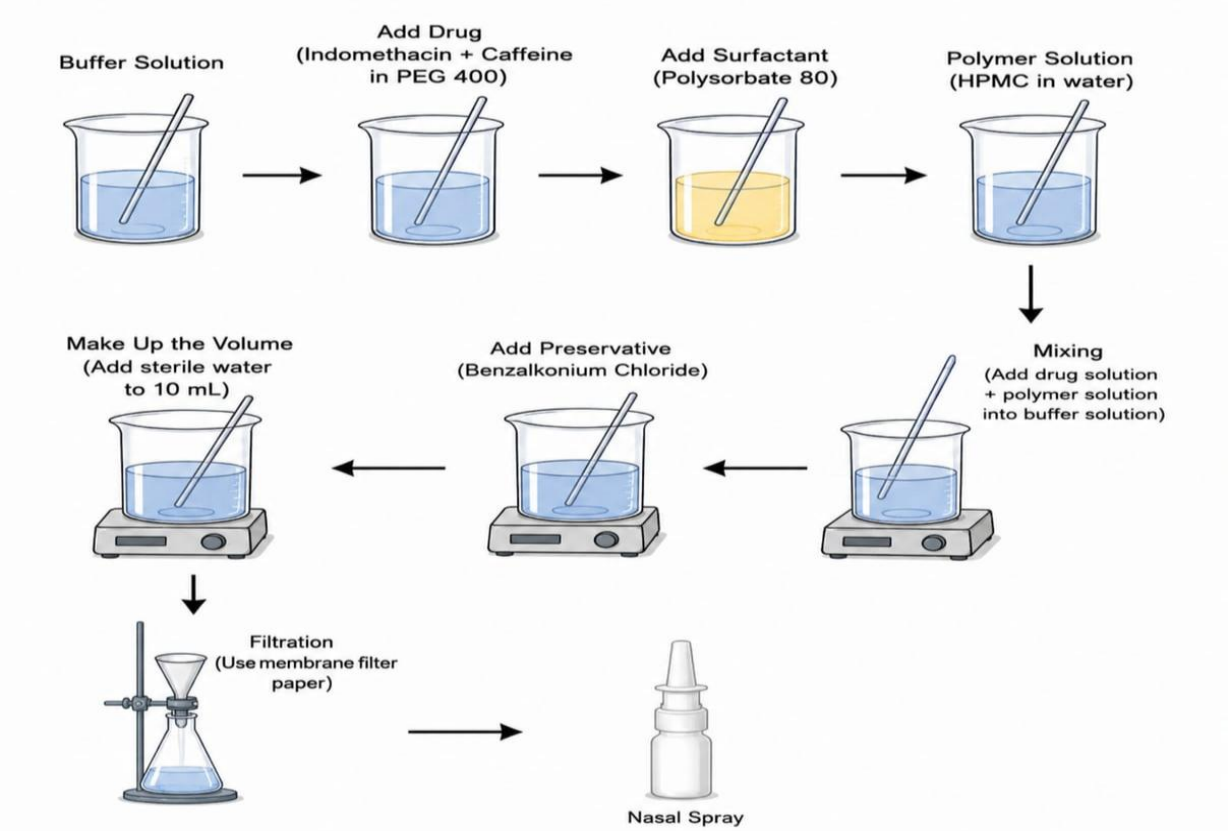


Figure 4: Method of preparation

Evaluation Tests

1. Physical Appearances

The formulated nasal drop was visually evaluated for colour, odour, and appearance. The colour of the nasal drop is colourless, and its odour is pungent.

2. pH test

The pH of the nasal formulation is very important, mainly to avoid irritation of the nasal
mucosa, to prevent the growth of pathogenic microorganisms, and to sustain normal physiological ciliary movement. Lysozyme, present in nasal secretions, is responsible for destroying certain microorganisms at acidic pH. Under alkaline pH, Lysozyme is deactivated, and the nasal tissue is susceptible to microbial infection. It is therefore the pH of the formulation was adjusted between 4.5 and 6.5, and the pH of all the prepared formulations was measured using a digital pH meter.

Procedure:

Calibrate a digital pH meter using standard buffers (pH 4,7). Take the nasal spray sample. Dip the electrode into the solution. Record the pH.

Ideal Range: 4.5 – 6.5 (suitable for nasal mucosa)

3. Sterility

The test for sterility was designed to reveal the presence of microorganisms in the colloidal dispersions. Soya bean casein digested media was used in this study in order to find out both bacteria and fungi. One portion of the intended media was used for the detection of bacteria at 37 °C for 24h, and another portion was used for the detection of fungi at 23 °C for seven days.

Procedure:

Place the sample to be sterilised in the autoclave. The door is locked. A vacuum pump sucks the air from the chamber. The temperature reaches 121 °C for 30 minutes.

4. Pump delivery

The formulation was filled into a container having a single nozzle (0.2 mm diameter) and was actuated 10 times in a pre-weighed weighing bottle. After actuation, the weight of the weighing bottle was reweighed, and the difference was calculated.

Procedure: Fill the nasal spray formulation into the container. Prime the pump properly before testing. Weigh the container before spraying. Actuate the spray a fixed number of times (usually 10 sprays). Weigh the container again after spraying. Calculate the amount delivered per spray.

Formula: $W_1 - W_2 / N$

Were,

W_1 is the initial weight of the bottle

W_2 is the final weight of the bottle

N is the no. of spray

5. Viscosity

For formulations containing an agent that contributes to viscosity, this parameter should be tested and controlled at release and during stability testing. The contact time between the drug and the nasal mucosa is increased by the formulation's higher viscosity, thereby prolonging permeation. Also, high viscosity of formulations interferes with normal ciliary beating and/or MCC and thus, increases the permeability of drugs.

Procedure: Viscosity Evaluation Test for Nasal Spray. Take the prepared nasal spray formulation in a beaker. Measure viscosity using a Brookfield Viscometer at room temperature. Select a suitable spindle and rotation speed. Immerse the spindle properly in the formulation without air bubbles. Record viscosity reading in centipoise (cP). Repeat the reading three times and calculate the average viscosity.Ideal viscosity is 1-100cp

6. Droplet size distribution

Uniform droplets indicate good spray performance and proper atomization. The standard/optimum droplet size for nasal spray is generally:10-100um

Procedure: Shake the nasal spray properly. Spray once on a clean glass slide/filter paper from a fixed distance. Allow droplets to settle for a few seconds. Observe the slide under the microscope. Measure the diameter of different droplets using a stage micrometre. Record sizes of multiple droplets. Calculate average droplet size.

Formula: $\sum d / n$

Were,

- $\sum d$ = Sum of the droplet diameters
- n = No. of the droplet measured

V. RESULT

1. Physical Appearance

- Colour: Pale yellow
- Odour: Pungent

2. Ph Test:

Sample	pH value
F1	5.5
F2	5.6
F3	5.7
F4	6.0

Table 2: pH test

The mean pH value of the sample was found to be 5.7

Figure 5: PH Test

Sterility Test

There would be no Microbial growth occurred in the media culture after the incubation period of 14 days. Hence, the sample passed the test.

Figure 6: Sterility test

3. Viscosity Test:

"The ideal range of viscosity for nasal spray 1–100 cP, indicating a suitable flow property for nasal spray administration."

"Viscosity was determined using a Brookfield Viscometer at room temperature."

Formulation	viscosity
F1	1.40cp
F2	1.41cp
F3	1.46cp

Table 3: Viscosity Test

The viscosity was found to be 1.42 cp.

Figure 7: viscosity test

4. Pump delivery: The standard pump delivery of nasal spray generally ranges between

0.05–0.15 mL/spray.

To calculate the amount of drug delivered per spray.

Formula: $W_1 - W_2 / N$

Were, 40

Initial weight of the bottle $= W_1 = 40.69$g.

Final weight of the bottle $= W_2 = 40.60$g.

The no. of spray $= N = 1$

Therefore, Pump delivery per spray = 40.69g – 40.60g / 1

= 0.09g/spray.

The pump delivery of the nasal spray formulation was found to be 0.09g/spray or 0.09ml/spray, which is within an acceptable range for a nasal spray.

5. Droplet Size distribution: To measure the droplet sizes of the nasal spray

Spray No.	Droplet Size (µm)
1	32
2	35
3	30
4	34
5	33

Table 4: Droplet size distribution

Formula: Average Droplet Size $= \sum d/n$

Where:

- $\sum d$ = Sum of droplet diameter
- N = No. of droplets measured

Calculation

Avg of Droplet size = 32+35+30+34+33 /5

=164/5

Avg of Droplet size = 32.8 µm

VI. CONCULSION

All the sample drugs and their excipients used were as per the standardsThe prepared formulation was evaluatedThe physical evaluation parameters like colour, odour, pH test, viscosity test, pump delivery test and sterility test were found to be satisfactorySo it can be concluded that the formulation had a better pH and viscosity valueThe bioavailability of the formulation was high due to no hepatic first-pass metabolismThe formulation was found to be stable at room temperature.

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