FORMULATION AND EVALUATION OF ANTIEMETIC NASAL SPRAY OF LIMONENE

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ABSRACT

The objective of present investigation was to isolate the phytochemical component, limonene from the peels of Citrus sinensis belonging to family Rutaceae and formulate anti-emetic nasal spray. Limonene loaded colloidal dispersion was prepared by using various polymers like HPMC, DSHOP, and surfactant as Tween 80. Further the formulation was evaluated for its clarity, pH, viscosity, pka, pump delivery, net content, and droplet diameters. Limonene was obtained by steam distillation method and subjected to its phytochemical screening and Pharmacopoeial procedures were used to study the solubility, organoleptic properties, pH, viscosity, pka , pump delivery, net content, droplet diameters. The pH of the formulation was adjusted between 5.0-5.5 which is nonirritant for nasal delivery system.

The result showed that the optimized formulation was found to be colorless, orange like odor and shows adhesive property. The adhesive property in the formulation is because of the presence of mucoadhesive polymer, which prolongs the effectiveness of dosages form by its mucoretentive effect onto the nasal mucosa. To evaluate the spray related property, a plastic spray container was selected and evaluated for the release of net content which was found to be 20 ml and total spray pattern of the formulation was found to be 322 sprays. The above study shows that the limonene loaded antiemetic formulation can be given via nasal route to enhance the effectiveness as well as onset of action.

KEYWORDS: Limonene, HPMC, DSHOP, Steam distillation method, mucoadhesive polymer, mucoretentive effect.
INTRODUCTION
Nasal drug delivery has been recognized as a very promising route for delivery of therapeutic compounds including biopharmaceuticals. Nasal administration is a logical choice for topical nasal treatments such as antihistamines and corticosteroids. The nasal mucosa has also received attention as a viable means of systemic administration of analgesics, sedatives, hormones, cardiovascular drugs, and vaccines. Conventionally, the nasal route has been used for local delivery of drugs for treating nasal allergy, nasal congestion, or nasal infections. However systemic delivery through the nasal route has recently begun to explore possibilities for those requiring a rapid onset of action or necessitating avoidance of severe proteolysis involved in oral administration (e.g., most peptide and protein drugs). Successful attempts to deliver corticosteroid hormones through the nasal route for systemic absorption have triggered further studies in this area.

Researchers have studied the anatomical and physiological aspects of the nasal membrane, including its vascular nature, as they relate to drug delivery. There are three distinct functional regions in the nose- the vestibular, respiratory, and olfactory. Among these, the respiratory region is the most important for systemic drug delivery. The respiratory epithelium consists of basal, mucus-containing goblet, ciliated columnar and non-ciliated columnar cell types. The cilia move in a wavelike fashion to transport particles to the pharynx area for ingestion. Additionally, the cells in this region are covered by nearly 300 microvilli, providing a large surface area for absorption. Below the epithelium is the lamina propria. This is where blood vessels, nerves, serous glands, and mucus secretory glands may be found. The lamina propria also houses a dense network of capillaries, through which drug absorption takes place. The nasal passage epithelium is covered by a mucus layer that is renewed every 10 to 15 minutes. The pH of the mucosal secretions ranges from 5.5 to 6.5 in adults and 5.0 and 6.7 in children. The mucus layer entraps particles, which are then cleared from the nasal cavity by the cilia.

Nausea and vomiting are features of many GI and non-GI diseases and disorders. Regardless of its cause, treatment of nausea and vomiting should initially focus on replacing volume and electrolyte deficits. Later on, nutritional deficits must be addressed. Regardless of its cause, nausea and vomiting can cause several life-threatening GI and non-GI complications. Elucidation of the cause is often possible, and treatment of the underlying cause will usually
be successful. Effective symptomatic therapies for nausea and vomiting are available when the cause is unclear or when the treatment of the underlying cause takes time to work.[1]

Advantages with nasal systemic drug delivery: The nasal cavity is covered by a thin mucosa which is well vascularised. Therefore, a drug molecule can be transferred quickly across the single epithelial cell layer directly to the systemic blood circulation without first-pass hepatic and intestinal metabolism. The effect is often reached within 5 min for smaller drug molecules. Nasal administration can therefore be used as an alternative to oral administration of for example tablets and capsules if a fast effect is desired or if the drug is extensively degraded in the gut or liver, drug which shows poor absorbability can be given by this route.

Limitations with nasal systemic drug delivery: Nasal administration is primarily suitable for potent drugs since only a limited volume can be sprayed into the nasal cavity. Drugs for continuous and frequent administration may be less suitable because of the risk of harmful long term effects on the nasal epithelium. Nasal administration has also been associated with a high variability in the amount of drug absorbed. Upper airway infections may increase the variability as may the extent of sensory irritation of the nasal mucosa, differences in the amount of liquid spray that is swallowed and not kept in the nasal cavity and differences in the spray actuation process. However, the variability in the amount absorbed after nasal administration should be comparable to that after oral administration.[2]

NASAL ANATOMY AND PHYSIOLOGY OF THE NOSE

Fig-Anatomy Of Nose

The human nasal cavity has a total volume of about 16 to 19 ml, and a total surface area of about 180 cm², and is divided into two nasal cavities by the septum. The volume of each
cavity is approximately 7.5 ml, having a surface area approximately 75 cm². Post drug administration into the nasal cavity, a solute can be deposited at one or more of anatomically distinct regions, the vestibular, respiratory and olfactory regions showing in figure.

MECHANISM OF NASAL ABSORPTION

The absorbed drugs from the nasal cavity must pass through the mucus layer; it is the first step in absorption. Small, unchanged drugs easily pass through this layer but large, charged drugs are difficult to cross it. The principle protein of the mucus is mucin; it has the tendency to bind to the solutes, hindering diffusion. Additionally, structural changes in the mucus layer are possible as a result of environmental changes (i.e. pH, temperature, etc.) (Ileum et al, 1999). So many absorption mechanisms were established earlier but only two mechanisms have been predominantly used, such as

First mechanism

It involves an aqueous route of transport, which is also known as the paracellular route but slow and passive. There is an inverse log-log correlation between intranasal absorption and the molecular weight of water-soluble compounds. The molecular weight greater than 1000 Daltons having drugs shows poor bioavailability (Aurora, 2002).

Second mechanism

It involves transport through a lipoidal route and it is also known as the transcellular process. It is responsible for the transport of lipophilic drugs that show a rate dependency on their lipophilicity. Drug also cross cell membranes by an active transport route via carrier-mediated means or transport through the opening of tight junctions.

![Diagram](image-url)

**Fig (A1) Intercellular spaces, (A2) Tight junctions, (B1) Passive diffusion, (B2)**
Active transport, (C) Transcytosis

Drugs for nasal administration

Nasal sprays for local effect are quite common. Several antimigraine drugs are also currently administered by nasal administration because a fast effect is desired and oral administration can be prohibited by nausea. Peptide drugs (hormone treatments) are also available as nasal sprays, in this case to avoid drug degradation after oral administration. The peptide analogue desmopressin is, for example, available for both nasal and oral administration. The bioavailability of the commercial tablet is 0.1% while that of the nasal spray is 3-5% according to the SPC (Summary of Product Characteristics). Other potential drug candidates for nasal administration include anaesthetics, antiemetic and sedatives that all benefit from a fast onset of effect.

MECHANISM OF EMESIS

- **Nausea**: from the Latin nausea (a ship); a very unpleasant sensation that one may soon vomit
- **Vomiting**: involuntary contractions of the abdominal, thoracic and GI (smooth) muscles leading to forceful expulsion of stomach contents from the mouth.

About Drug- Limonene\(^{[5]}\)

Limonene is found to be an antiemetic agent and is a colourless liquid hydrocarbon classified as a cyclic terpene. The more common D isomer possesses a strong smell of oranges.\(^{[1]}\) It is used in chemical synthesis as a precursor to carvone and as a renewably based solvent in cleaning products.
Limonene takes its name from the lemon, as the rind of the lemon, like other citrus fruits, contains considerable amounts of this compound, which contributes to their odour. Limonene is a chiral molecule, and biological sources produce one enantiomer: the principal industrial source, citrus fruit, contains D-limonene ((+)–limonene), which is the (R)-enantiomer (CAS number 5989-27-5, EINECS number 227-813-5). Racemic limonene is known as dipentene. D-Limonene is obtained commercially from citrus fruits through two primary methods: centrifugal separation or steam distillation.

**Biosynthesis:** Limonene is formed from geranyl pyrophosphate, via cyclization of a neryl carbocation or its equivalent as shown. The final step involves loss of a proton from the cation to form the alkene.

The most widely practiced conversion of limonene is to carvone. The three step reaction begins with the regioselective addition of nitrosyl chloride across the trisubstituted double bond. This species is then converted to the oxime with base, and the hydroxylamine is removed to give the ketone-containing carvone.

**Uses:** Limonene is common in cosmetic products. As the main odour constituent of citrus (plant family Rutaceae), D-limonene is used in food manufacturing and some medicines, e.g. as a flavouring to mask the bitter taste of alkaloids, and as a fragrant in perfumery; it is also used as botanical insecticide, particularly the (R)-(+)–enantiomer is most active as an insecticide. It is added to cleaning products such as hand cleansers to give a lemon-orange fragrance (see orange oil). In contrast, L-limonene has a piney, turpentine-like odour.

In natural and alternative medicine, D-limonene is marketed to relieve gastroesophageal reflux disease and heartburn. Limonene is increasingly being used as a solvent for cleaning purposes, such as the removal of oil from machine parts, as it is produced from a renewable source (citrus oil, as a by product of orange juice manufacture). It also serves as a paint...
stripper when applied to painted wood and is also useful as a fragrant alternative to turpentine. Limonene is also used as a solvent in some model airplane glues. All-natural commercial air fresheners, with air propellants, containing limonene are used by philatelists to remove self-adhesive postage stamps from envelope paper. As it is combustible, limonene has also been considered as a biofuel. Limonene can be used to dissolve polystyrene, and is a more ecologically friendly substitute for acetone. In preparing tissues for histology or histopathology, D-limonene is often used as a less toxic substitute for xylene when clearing dehydrated specimens. Clearing agents are liquids miscible with alcohols (such as ethanol or isopropanol) and with melted paraffin wax, in which specimens are embedded to facilitate cutting of thin sections for microscopy.

EXPERIMENT[6]

Extraction Of Limonene From Orange Peel

The peel of oranges was boiled in water and the oil produced (limonene) distilled in steam at a temperature just below 100 °C, well below its normal boiling point. The immiscible oil can then be separated. Direct extraction by heating would result in decomposition whereas steam distillation does not destroy the chemicals involved. The experiment also links for tests for unsaturation, and at a higher level, chirality.

Stage 1

- Grate the outer orange coloured rind of oranges and add to 100 cm$^3$ of distilled water in the 250 cm$^3$ round bottomed flask. Add anti-bumping granules to the round bottomed flask.
- Set up the distillation apparatus as shown in the apparatus section.
- Heat the flask so that distillation proceeds at a steady rate, approximately one drop per second of distillate. (Note: Take care not to let the liquid in the round bottomed flask boil too strongly).
- Collect approximately 50 cm$^3$ of distillate in the measuring cylinder. The oil layer will be on the surface.
- Using a dropping pipette carefully remove the oil layer into a test tube for the next stage.

Stage 2

- Cautiously smell the extracted oil by wafting the fumes towards the nose. Do not breathe in directly from the test tube

**Preparation of Antiemetic Nasal Spray using Limonene**$^7$

To formulate a nasal spray definite amount of limonin was added to aqueous solution containing citric acid, disodium hydrogen ortho phosphate, dextrose, methyl peraben, propyl peraben, tween 80, HPMC. It was stirred for 30 minutes and temperature cooled down up to 50°C to obtaining clear solution and then volume was make up by remaining water.

Note*: Tween 80 is used as emulsifying agent so that the oil droplets mixed properly with the aquous phase.

**Table 6.1 Formulation Of Nasal spray**

<table>
<thead>
<tr>
<th>S.No</th>
<th>INGREDIENTS</th>
<th>Amount(v/v)% 50 ml</th>
<th>Amount(v/v)% 100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Limonene-(Drug)</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Dextrose-(Tonicity)</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>HPMC-(Viscosity inercher)</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>DSHOP-(Buffering agent)</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>5</td>
<td>Citric acid-(Buffering agent)</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>6</td>
<td>Methyl paraben-(Preservative)</td>
<td>0.14</td>
<td>0.28</td>
</tr>
<tr>
<td>7</td>
<td>Propyl paraben-(Preservative)</td>
<td>0.14</td>
<td>0.28</td>
</tr>
<tr>
<td>8</td>
<td>Phenyl alcohol</td>
<td>0.15</td>
<td>0.30</td>
</tr>
<tr>
<td>9</td>
<td>Tween 80-(Emulsifying agent)</td>
<td>0.15</td>
<td>0.30</td>
</tr>
</tbody>
</table>

**DELIVERY SYSTEM**$^8$

**NASAL SPRAY**-

Nasal drops and liquid nasal sprays are solutions, emulsions or suspensions intended for instillation or spraying into the nasal cavities. Emulsions may show evidence of phase separation but are easily redispersed on shaking. Suspensions may show a sediment which is readily dispersed on shaking to give a suspension which remains sufficiently stable to enable
the correct dose to be delivered. Nasal drops are usually supplied in multidose containers provided with a suitable applicator. Liquid nasal sprays are supplied in containers with atomising devices or in pressurised containers fitted with a suitable adapter and with or without a metering dose valve, which comply with the requirements of the monograph on Pressurised pharmaceutical preparations (0523). The size of droplets of the spray is such as to localise their deposition in the nasal cavity.

Nasal Spray

**RESEARCH ENVISAGED**

Intranasal delivery of medicaments can achieve various therapeutic advantages. This route has very high penetration through mucous membrane. It is used as the delivery of drug through nose to brain. During travelling vomiting is a big problem in most of the people. Limonene obtained from orange peel gives relief by emesis; most of the people take oranges because in oranges a aromatic oil is found whose smell gives relief from vomiting nd this is due to limonene which inhibits CTZ due to its aroma which goes to brain via nose.

**EVALUATION PARAMETERS**

*Appearance, Colour & Clarity*- The appearance of the content of the container (i.e., formulation) and the container closure system (e.g., pump components, inside of the container) should conform to their respective descriptions as an indication of the drug product integrity. If any colour is associated with the formulation (either present initially or from degradative processes occurring during shelf life) then a quantitative test with appropriate acceptance criteria should be established for the drug product by the manufacturer.

*pH*- For both solution and suspension nasal sprays, the apparent pH of the formulation should be tested and an appropriate acceptance criterion established. Lysozyme is found in nasal secretions, which is responsible for destroying certain bacteria at acidic pH. Under alkaline conditions, lysozyme is inactivated and the nasal tissue is susceptible to microbial infection.
It is therefore advisable to keep the formulation at a pH of 4.5 to 6.5 keeping in mind the terms of its sensitivity and ability to detect shifts that may occur in the distribution. The acceptance criteria should control the complete distribution and should reflect the data obtained for the submitted batches (e.g., clinical, preclinical, biobatch, primary stability, production).

**Pump Delivery** - A test to assess pump-to-pump reproducibility in terms of drug product performance and to evaluate the metering ability of the pump should be performed. The proper performance of the pump should be ensured primarily by the pump manufacturer, who should assemble the pump with parts of precise dimensions. Pump spray weight delivery should be verified by the applicant for the drug product. In general, pump spray weight delivery acceptance criteria should control the weight of the individual sprays to within ±15 percent of the target weight and their mean weight to within ±10 percent of the target weight.

**Foreign Particulates** - For both solution and suspension nasal sprays, there should be validated tests and associated acceptance criteria for foreign particulates. Foreign particulates may originate during manufacturing, from formulation components, and, in particular, from the container and closure components. Levels of foreign particulates in the drug product may increase with time, temperature, and stress.

**Droplet Diameter** - For both suspension and solution nasal sprays, the specifications should include an appropriate control for the droplet size distribution (e.g., 3 to 4 cut-off values) of the delivered plume subsequent to spraying under specified experimental and instrumental conditions. Appropriate and validated dynamic plume droplet size analytical procedures should be described in sufficient detail to allow accurate assessment by Agency laboratories (e.g., apparatus and accessories, software version and calculation algorithms, sample placement, laser trigger condition, measurement range, beam width).

**Net Content and Weight Loss (Stability)** - Nasal spray drug products should include acceptance criteria for net content and weight loss on stability. Since storage orientation plays a key role in any weight loss, the drug product should be stored in upright and inverted or upright and horizontal positions to assess this characteristic. The total net content of all formulation components in the entire container should be determined. The net content of each of 10 test containers should be in accordance with the release specification.
Adhesiveness—Proper adhesiveness is very much essential for better drug release because it exerts its effect on drug contact with nasal mucosa which directly effects the therapeutic effect of nasal drug delivery system and it is measured by measuring flow volume of the solution through glass surface (kept in 45°) and its adhesion to large surface.

RESULTS

<table>
<thead>
<tr>
<th>S. No</th>
<th>Evaluation Parameters</th>
<th>Formulation 1</th>
<th>Formulation 2</th>
<th>Formulation 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Colour</td>
<td>Milky White</td>
<td>White</td>
<td>Clear</td>
</tr>
<tr>
<td>2</td>
<td>Odour</td>
<td>Phenolic</td>
<td>Orange Like</td>
<td>Orange Like</td>
</tr>
<tr>
<td>3</td>
<td>pH</td>
<td>6.0</td>
<td>6.0</td>
<td>5.0</td>
</tr>
<tr>
<td>4</td>
<td>Clarity</td>
<td>Clear</td>
<td>Clear</td>
<td>Clear</td>
</tr>
<tr>
<td>5</td>
<td>Adhesiveness(%)</td>
<td>2.5%</td>
<td>2.5%</td>
<td>2%</td>
</tr>
<tr>
<td>6</td>
<td>Droplet Diameter-</td>
<td>2 cm</td>
<td>1.9 cm</td>
<td>1.5 cm</td>
</tr>
<tr>
<td>7</td>
<td>Pump Delivery(in 20 ml)</td>
<td>300 sprays</td>
<td>320 sprays</td>
<td>322 sprays</td>
</tr>
</tbody>
</table>

CONCLUSION

Nasal drug administration here is used as delivery of drug via nose to brain and shows therapeutic effect in the prevention of vomiting and shows better therapeutic efficacy over other dosage forms. The optimized formulation was colourless, orange like smell and clear along with proper adhesiveness with good release profile. Spray related properties were also evaluated. To perform spray related properties a plastic spray container was selected and evaluated for net content which was obtained 20 ml. Total spray pattern of the formulation was found to be 322 sprays, ph was found to be 5.0 and the droplet diameter was found to be 1.5 cm.

REFERENCES