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Review Article

Intranasal In-Situ Gelling Systems: An Approach for Enhanced CNS Delivery of Drugs

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Abstract

The oral route is the one that is most frequently used for drug administration. Unfortunately, this oral route is not the best for the delivery of several medications because of gastrointestinal breakdown and substantial hepatic first-pass metabolism. As an alternative, the nasal route can be chosen to deliver drugs via the olfactory and trigeminal neurons directly to the brain bypassing the blood-brain barrier (BBB). The advantages of the nasal route are its non-invasiveness, and self-medication. The main drawback of this route is the quick mucociliary clearance, which leads to low absorption and consequently poor bioavailability. This drawback can be overcome by adopting in situ mucoadhesive gelling systems. The in-situ gelling systems are liquids that upon administration turn into gel as a result of various physiological stimuli, such as temperature or pH or ionic. In addition to discussing the polymers employed in the formulation of in situ gels, approaches of in situ gelation, mechanism of gelation, and their evaluation, the current review critically assesses the significance of in situ gelling systems for the delivery of medications from the nose to the brain.

Keywords: Nose-to-brain Delivery, CNS Targeting, Stimuli-Responsive Polymers, Intranasal Delivery, In-Situ Gelation.

Introduction

When systemic effects are desired, the oral route is the most desired and convenient method for administration of drugs. Unfortunately, this oral route is not the best for the delivery of several medications because of gastrointestinal breakdown and substantial hepatic first-pass metabolism which evokes the search for new routes to deliver these drugs. The parenteral route, transdermal route, and transmucosal routes are versatile and allow the delivery of various drugs. One of the main advantages of parenteral and transmucosal routes of drug delivery is that they bypass the first-pass effect, can reduce the dose and improve the safety of the drugs and reduce the overall treatment cost. One of the main disadvantages of the parenteral route is that it can cause pain at the site of injection. This can be very inconvenient for patients to continue taking the medication for a long time. Furthermore, because of the limited permeability of the skin, the transdermal route, while successfully utilized for the delivery of some medications, is less efficient than the oral route. The problem with choosing the rectal and vaginal routes is that they cause irritation. In the buccal route, the

unpleasant taste of the drugs can cause a problem with acceptability. Thus, Intranasal drug delivery is considered a promising choice in contrast to the oral route due to its potential to overcome various major limitations. [1]

In the past, nasal medication delivery was employed for localized treatment. Studies conducted over the last three decades have demonstrated that this method is likewise a reliable and effective means of delivering medications to the systemic route. Proteins, peptides, different polar drugs with small molecular weights, and macromolecules like vaccines and DNA which are highly digested or incompletely absorbed can all be administered via the nasal route because of its rich vasculature and high drug permeation rate. Intranasal drug delivery provides a concentration-time profile similar to that of intravenous administration. This allows for the quick onset of action of the drugs given by the nasal route. [1]

Furthermore, using pathways through the olfactory and trigeminal nerves nasal route offers a non-invasive alternative bypassing the blood-brain barrier (BBB) in order to deliver drugs directly to the CNS.[1,2]

Even while intranasal delivery has its own benefits, some formulations are still not suitable because of their limited permeability and short residence times. Rapid mucociliary clearance (MCC) is one of the main causes of this problem which can be minimized by making the formulation more viscous and mucoadherent. These can facilitate the absorption of the medication and prolong the duration that a drug remains at the nasal absorption site. Unfortunately, the high viscosity of the solution makes it challenging to administer and dose the medication accurately. Consequently, the need to meet these unique difficulties lead to the invention of an intranasal in-situ gelling systems.[2]

Insitu Gel

In-situ is a Latin phrase that translates to “on-site” or “in place” or “on the premises.”[3] A gel is a transitional state between a liquid and a solid composed of networks of long polymer molecules that are physically cross-linked, with liquid molecules trapped within a three-dimensional polymeric network inflated by a solvent.

These nasal In-situ gels are made from low-viscosity biocompatible materials which are in liquid form and upon interaction with the nasal mucosa, the polymer undergoes structural changes making a gel so that it is able to improve the interaction between the drugs and the nasal mucosa. They can also release the drugs slowly in the nasal cavity in reliable quantities making it more accurate.[4] The process of gelation can be achieved through the crosslinking of the various components of the polymer chain by the formation of non-covalent (physical) or covalent (chemical) bonds. There are various mechanisms that can be utilized to develop in-situ gel systems like physiological stimuli (such as temperature changes and pH-triggered systems), physical changes in the biomaterials (such as Diffusion, swelling, and osmosis), and chemical interactions (e.g. UV radiation, enzymatic and ion activated systems).[4,5]

Nasal Drug Delivery System:

Intranasal delivery is a viable alternative to the conventional routes of drug delivery. The nasal cavity can be used as a site of administration for the local and systemic delivery of different pharmaceuticals, according to numerous research. Treatment of localized, systemic, and CNS sites are possible with intranasal administration.[4]

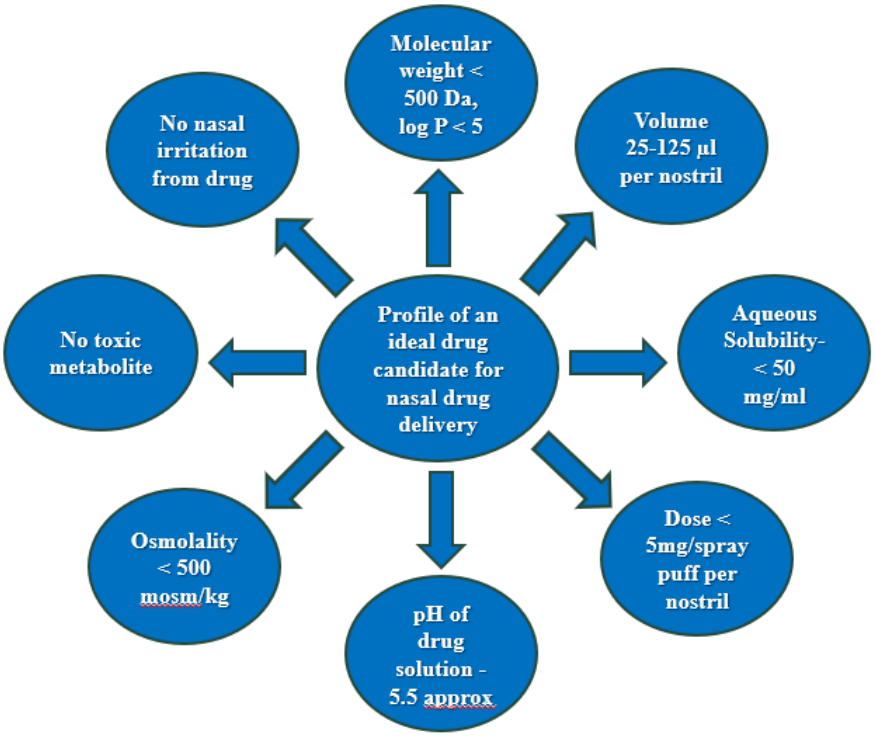

Figure 1: Gives the characteristics of the appropriate drug candidate suitable for nasal drug administration.[1]

ANATOMY AND PHYSIOLOGY OF NOSE

Breathing and olfaction are the two major activities of the nasal cavity. It's own a surface area of about 150 cm^2, and its total volume is somewhere between 15 and 20 ml. The nasal septum divides it into two cavities. A mucus membrane made of mucus covers the nose canal epithelium. For every 20 minutes, the nasal mucous, which has a pH range of 5.0 to 6.5 and flows at a velocity of 5 to 6 mm/min, clears the nasal particles. This process is known as mucociliary clearance (MCC).[6]

Each part has three distinct regions namely,

1. Respiratory region: The conchae, also known as the respiratory region, is the biggest portion of the nasal cavity. It is crucial for the delivery of medications systemically. It is made up of four main cell types: goblet cells, ciliated columnar cells, non-ciliated cells, and basal cells. The superior, middle and inferior nasal turbinates protrude from the lateral walls of each nasal cavity.

2. Vestibular region: The nasal vestibule has a surface area of approximately 0.6 cm and is situated right inside the nostrils. A stratified squamous and keratinized epithelium covers this area. The airborne particles are filtered out by the sebaceous glands that are located in this area.

3. Olfactory region: The nasal cavity's roof is site of the location of olfactory region, which has a surface area of around 10 cm^2. It dips into the lateral wall and septum for a short distance. It is crucial for the delivery of medications to the central nervous system and cerebrospinal fluid.[4,6,7]

Pathways:

The olfactory and trigeminal neurons in the brain are thought to be the major neuronal pathway for the delivery of drug form nose to brain with the CSF, the lymphatic system, and vascular absorption (through general circulation) being secondary pathways.

1. Pathway utilizing olfactory and trigeminal neurons: The primary and direct route of medication absorption from the nasal to the brain region is through the olfactory and trigeminal nerves. The first method entails an intracellular axonal transport of the medication to the primary neurons of the olfactory epithelium and eventually to the olfactory bulb. Later subsequent distribution into further distant brain tissues. The second mechanism relies on drug uptake into the CNS after passing through the olfactory sustentacular epithelium cells. Passive diffusion, endocytosis, or paracellular transport are the three mechanisms used for drug transport through olfactory neurons.

The trigeminal nerve, on the other hand, creates a connection between the nasal cavity and the cerebrum and pons regions of the brain, as well as, a lesser extent, to the frontal cortex and olfactory bulb. So, one can predict the brain's target place by carefully comprehending the mechanics of drug transport. Yet, managing transportation along a single route is quite difficult.

2. Pathway utilizing the CSF & lymphatic system:

Olfactory nerves located in the perineural space are responsible for drug absorption to the CSF of the subarachnoid space of the CNS through the lymphatic system of the nasal cavity. The medication first reaches the CSF and perivascular area in this pathway before distributing to the remaining areas of the brain. The drug transportation and distribution in the CSF depends on lipophilicity, polarity, solubility, degree of ionization, and molecular weight.

3. Vascular absorption (through general circulation):

When a medication is administered more deeply into the nasal cavity, some quantity of the drug also reaches the systemic circulation through the respiratory region's vasculature and then travels to the brain as per the blood volume distribution after crossing the BBB (mostly lipophilic drugs).[7-10]

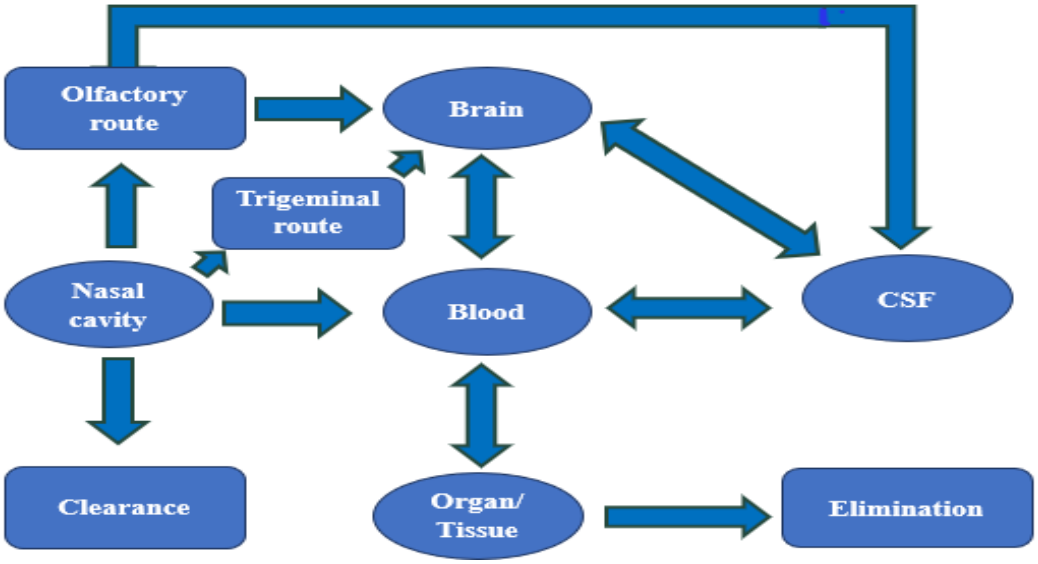

Figure 2: Illustrates a different drug delivery pathways form nose-to-brain.[7]

Challenges/Barriers To Nasal Drug Delivery: Below is a discussion of some key attributes of several barriers that influence nasal drug delivery:

Poor permeation and low bioavailability of drugs: A thin layer of mucus with built-in lipophilicity lines the nasal cavity. Moreover, the main drug delivery method from nose to brain relies on cellular transport, which only permits small lipophilic molecules to pass through. Hence, limiting the entry of polar molecules, the nasal route is more suited for delivering smaller size, lipophilic moieties.

Mucociliary clearance and poor drug retention: Mucociliary clearance is the result of the nasal cavity's cilia and mucus working together to prevent foreign particles from entering the body. The clearance lasts for 15 to 20 minutes. Drug absorption is constrained by both the quick evacuation and the poor drug retention in the nasal cavity.

Enzymatic degradation: The peptide/protein-based bio actives are broken down and their bioavailability is reduced by the exopeptidase and endopeptidase enzymes found in the epithelium and lumen of the nasal cavity.

Nasomucosal toxicity: The majority of drugs and excipients, in particular surfactants and organic solvents, have hazardous effects that can irritate or harm the nasal mucosa. In order to avoid harmful consequences, formulations intended for nose-to-brain administration should be safe for both the nasal mucosa and CNS.[9]

Advantages: [4,5,7,11,12]

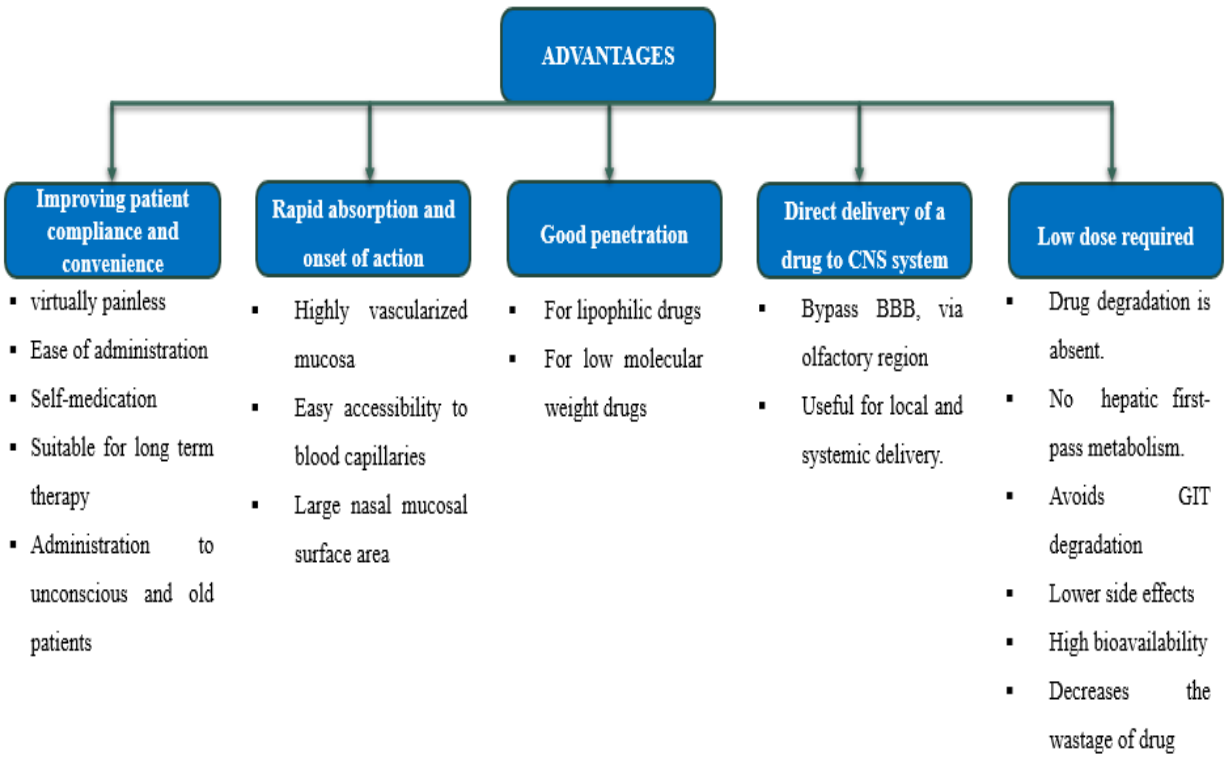

Figure 3: Advantages of in-situ gelling systems.

Limitation: [4,5,7,11,12]

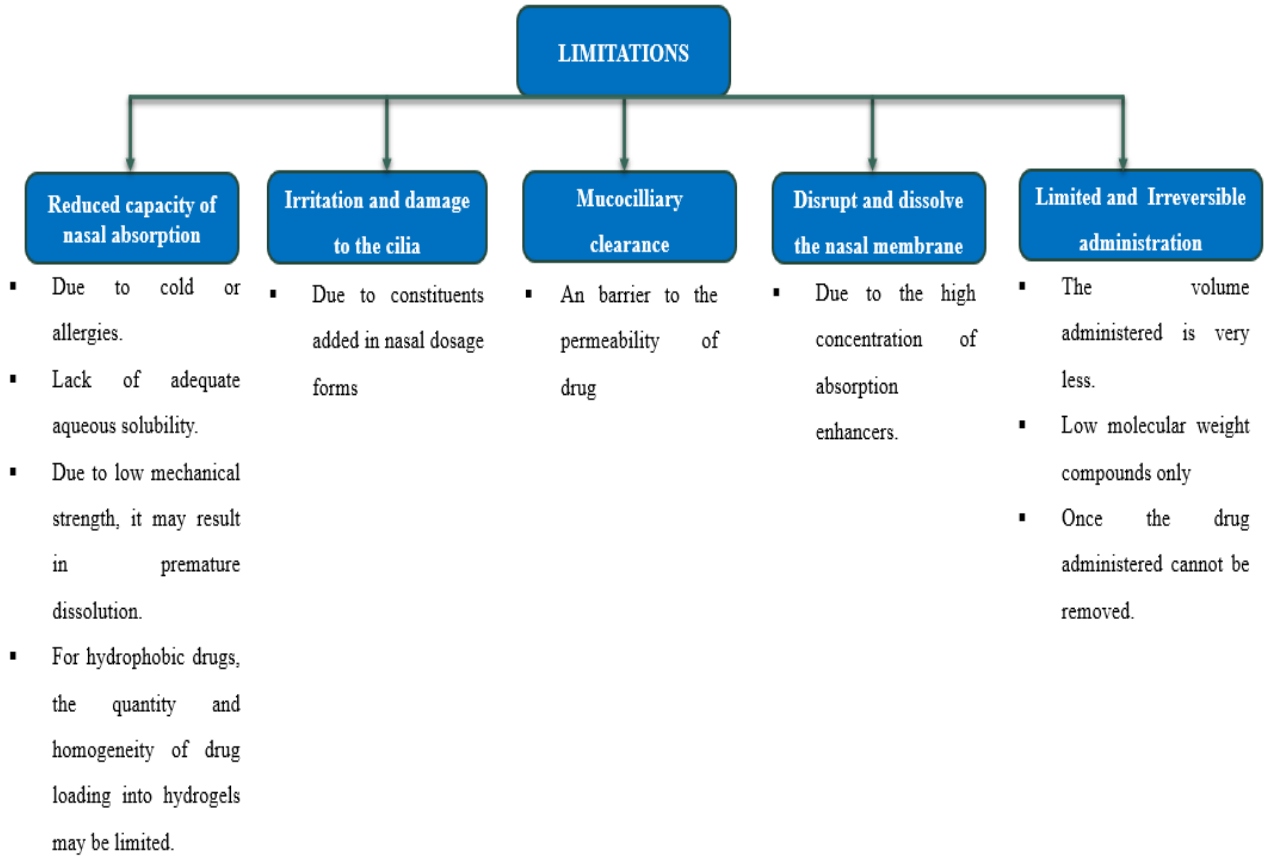

Figure 4: Limitations of in-situ gelling systems.[4,5,7,11,12]

Approaches of in Situ Gels:

There are three common pathways that induce the in-situ gels development of biomaterial:

A. **Physiologically induced In-Situ Gelling System** (e. g., Temperature and pH)
B. **Physically induced In-Situ Gelling System** (e. g., Diffusion, Osmosis and swelling)
C. **Chemical-induced In-Situ Gelling System** (e. g., enzymatic, chemical, and photo-initiated polymerization)

A. Physiological induced In-Situ Gelling System

Based on physiological stimuli in situ gelling systems are classified into two types:

Temperature triggered systems:

Gelling systems that are sensitive to temperature are frequently used stimuli-responsive systems. They do not require any external heat except body temperature to cause gelation. These in-situ gelling systems are in the liquid state at 20˚- 25˚C temperature and undergo gelation when coming in contact with body fluids at 35-37˚C. This is the easiest and most applicable strategy both in-vivo and in-vitro. Three main strategies are utilized in the design of these systems.

i. Negative thermo-sensitive type: e.g., poly-N-isopropyl acrylamide (PNiPAAm)

ii. Positive thermo-sensitive type: e.g., polyacrylic acid (PAA), or polyacrylamide, poly (acrylamide-co-butyl methacrylate).

iii. Thermo-reversible type; e. g. Pluronics (poloxamer), Tetronics (poloxamines),

cellulose derivatives (Methylcellulose, HPMC, EHEC and poly (ethylene oxide)-b-poly(propylene oxide).

pH triggered systems: A pH-sensitive gelling system can be created by the use of various polymers that are capable of reacting to the changing environmental pH. These polymers have ionizable functional groups that can easily lose or accept protons in response to the changing environmental pH. These polymers can be triggered by the presence of certain ionizable groups called poly-electrolytes. These groups can either increase or decrease the external pH, which leads to swelling and formation of the in-situ gels. Some of the anionic groups that are commonly used in the production of pH-sensitive gelling systems include the following: PAA (Carbopol, carbomer), polyethylene glycol, pseudo latexes, Polymethacrylic acid and cellulose acetate phthalate (CAP).

B. Physically Induced In-Situ Gelling System

These in-situ gelling systems are based on the notion that a material will swell and expand to fill the appropriate space as it absorbs water from its surroundings.

Diffusion or Solvent exchange: In this, solvent molecules diffuse into the surrounding tissue from the polymer solution. This process results in the solidification or precipitation of the polymer matrix. The commonly used polymer in this approach is N-methyl pyrrolidone (NMP).

Swelling: A certain material may swell and form an in-situ gel when it absorbs water from the surroundings. Myverol 18-99 (glycerol monooleate), which comprises polar lipid that expands in water and creates a lyotropic liquid crystalline phase structure, is one of the substances that can be employed in this method. It has bioadhesive properties and can be degraded in vivo.

Osmosis: A specific type of polymers that are responsive to changes in ionic strength are used in the osmotically induced gelling process. In the presence of mono- or divalent cations, the polymer's aqueous solution transforms into a transparent gel. It is considered that the rate of gelation can vary based on the osmotic gradient over the surface of the gel. Alginates and gellan gum are examples of polymers that exhibit osmotically induced gelation.

C. Chemically Induced In-Situ Gelling System: Precipitation of inorganic solids can occur as a result of chemical reactions that lead to in-situ gelation.

Ionic cross-linking: In the presence of different ions like k+, Ca2+, Na+, and Mg+2, ion-sensitive polymers such carrageenan, gellan gum, pectin, and sodium alginate undergo a phase transition to create gel.

Enzymatic cross-linking: The gel was created in this procedure by cross-linking with the enzymes found in the body fluid. Compared to chemical and photochemical approaches, this strategy has the advantage of functioning under physiological conditions without the use of potentially hazardous substances like monomers and initiators.

Photo-initiated polymerization: By infusing monomers or reactive micromere solutions, initiators, and electromagnetic radiation into a tissue location, in-situ gels are produced in this procedure. The most common polymers employed are those with long UV (such as ketones) and visible (such as camphor-quinone and ethyl eosin) wavelengths.[4,5,13]

Classification of in situ gel polymers

It is feasible to divide the polymers employed in in-situ gelling systems into two categories:

1. **Natural polymers** (E.g., Alginic acid, Gellan gum, Pectin, Chitosan,

Carrageenan, Xanthan gum, and Guar gum, etc.)

2. **Synthetic or semi-synthetic polymers** (E.g., poloxamers, Hydroxy propyl methyl cellulose, Cellulose acetate phthalate (CAP), Methylcellulose, polyacrylic acid, and poly (lactic-co-glycolic acid).

1. **Natural polymers**

a) **Alginic acid or sodium alginate:** Alginic acid is a linear block copolymer that is hydrophilic in nature. Aqueous solutions of alginates form gels upon the addition of di (Ca^{+2}, Mg^{+2}) and trivalent metal ions. Its favourable biological properties include its mucoadhesion, biodegradability, and nontoxicity.

b) **Carrageenan:** There are three different forms of carrageenan depending on the quantity and position of the sulphate group:

   i. **Iota carrageenan:** Iota carrageenan is totally soluble in hot water and, when combined with calcium or potassium ions, forms an elastic gel.

   ii. **Kappa carrageenan:** This substance resembles locust bean gum in that it is likewise soluble in hot water and gels when potassium ions are present.

   iii. **Lambda carrageenan:** In spite of being totally soluble in cold water, lambda carrageenan does not cause the development of gel. However it produces extremely viscous solutions.

c) **Chitosan:** Chitosan is a cationic, amino polysaccharide that can be formed by the alkaline deacetylation of chitin. It is a thermosensitive, biocompatible, and biodegradable copolymer that forms an in-situ gel by various stimuli such as ionic, temperature, and pH. Phosphate, oxalate, molybdate, and sulphate ions are responsible for the gelling of chitosan. Chitosan has been proven in enhancing the absorption of certain drugs by nasal delivery due to its high viscosity and bioadhesive nature.

d) **Gellan gum:** A gel is formed when an anionic deacylated gellan gum, such as alginate comes in contact with mono- or divalent metal cations. It is most frequently used polymer in the formation of in-situ gels. Given that the nasal mucosa's potassium, calcium, and sodium levels are sufficient to drive the gelation process, it is widely approved for nasal delivery due to its quick-gelling qualities.

e) **Pectin:** It is a cationic polysaccharide made up of methyl esters of -(1, 4)-D galacturonic acid. It only applies to formulations that are water soluble. The extent to which galacturonic acid has been esterified will impact how it gels. Because it is extremely mucoadhesive and capable of gelling upon contact with the nasal mucosa without the addition of exogenous cations, low methoxyl (LM) pectin is excellent for the delivery of drugs to the nasal cavity.

f) **Thiolated chitosan or Thiomers:** These are cationic, hydrophilic macromolecules, exhibiting much higher mucoadhesive properties compared to other polymers. It acts as a permeation enhancer. It interacts with mucus glycoproteins or cysteine-rich sub-domains by the simple oxidation process via crosslinking intra-and inter-disulphide bonds that lead to gel formation reaching the physiological environment.

g) **Xanthan gum:** It is a high-molecular weight polysaccharide that is soluble in both hot and cold water and exhibits good stability in acidic and alkali conditions.

2. Synthetic or semi-synthetic polymers

a) **Poloxamers:** These are commercially known as Pluronics. They undergo in situ gelation due to physiological

temperature. The concentration of polymer and PEO to PPO ratio influence the gelation temperature of the polymer. Among different grades, Pluronic F-127 is a commonly used in-situ gelling polymer that can be combined with other mucoadhesives such as HPMC and Carbopol 934 to ensure a long residence time on the site of application. It is also used to increase the rate of drug permeation across the mucosa. In addition, it can be used as a non-ionic surfactant to promote the absorption of drugs through the mucus by decreasing the elasticity and viscosity of the mucus.

b) **Hydroxypropyl methylcellulose (HPMC**) and **Methylcellulose (MC):** These are in-situ gelling cellulose derivatives that are biocompatible, thermoreversible, and mucoadhesive in nature. At temperatures above the physiological range, the aqueous solution of MC and HPMC undergoes a phase transition into gelling polymers, although this temperature can be decreased by modifying the polymers physically and chemically.

c) **Carbopol:** In comparison to other cellulose derivatives, this cross-linked polyacrylic acid has a high molecular weight and good mucoadhesive characteristics. At alkaline pH, it transforms into a low viscosity gel but remains in solution form at acidic pH. To form stiff gels a large concentration of Carbopol is required which is not easily neutralized by the buffering action of nasal mucus. A suitable polymer should be added to the formulation in order to enhance the gelling qualities and lower the overall polymer content.

d) **Poly (lactic-co-glycolic acid) or PLGA:** It is a synthetic copolymer of polyglycolic acid and polylactic acid that is biocompatible and biodegradable (PGA). In controlled drug delivery systems, these are employed.

e) **Poly (N-isopropyl acrylamide) or PNIPAAm:** It is a polymer which is temperature-sensitive and undergoes phase transition at 32–35°C, which is closer to the temperature of the human body.[1,2,12-15]

Table 1: In situ gelling systems

S.no	Drug	Category	Stimulus responsive agent	Triggering factor	Year	Ref
01	Doxylamine succinate and pyridoxine HCl	Gestational, nausea and vomiting	poloxamer407, poloxamer188, Carbopol 971P	Temperature	2022	[**16]**
02	Methotrexate	Anti-cancer	chitosan and Poloxamer 407	Temperature	2022	[**17]**
03	Granisetron	Anti-emetic	Poloxamer 407, Poloxamer 188 and Carbopol 971P	Temperature	2022	[**18]**
04	Darunavir	HIV infection	Poloxamer 407 and Carbopol 934P	Temperature	2022	[**19]**
05	Selegiline	Antidepressant	chitosan and ß-glycerophosphate	Temperature	2022	[**20]**

06	Mamentine HCl	Alzheimer's disease	Poloxamer-188 and Carbopol-934	Temperature	2021	[**21]**
07	Piribedil	Anti-Parkinson	Methyl Cellulose	Temperature	2021	[**22]**
08	Rufinamide	anti-epileptic	xyloglucan	Temperature	2021	[**23]**
09	Paroxetine	Antidepressant	Gellan gum and HPMC E15 LV	Ionic	2020	[**24]**
10	Sumatriptan succinate	Anti-migraine	Poloxamer 407 and HPMC K4 M	Temperature	2020	[**25]**
11	Duloxetine HCl	Antidepressant	F127, and Pluronic F68	Temperature	2020	[**26]**
12	lamotrigine	Anticonvulsant	sodium alginate, methyl cellulose and chitosan	pH	2019	[**27]**
13	Clonazepine	Anti-psychotic agent	Pluronic F-127 and F-68	Temperature	2019	[**28]**
14	Almotriptan maleate	Anti- migraine	Poloxamer 407	Temperature	2018	[**29]**
15	Buspirone HCL	anxiolytic agent	Carbopol 934P	pH	2018	[**30]**

Methods of preparation

Generally, in-situ gelling systems are prepared by two methods namely:

1. Cold Method and 2. Hot Method

1. **Cold method:** The cold method involves stirring a drug with enough distilled water and storing it overnight at 4°C in a refrigerator. The solution is then gradually supplemented with the in-situ gelling polymer while being continuously stirred. The mixture is then kept in a refrigerator until it transforms into a clear solution. The volume is finally adjusted. When chitosan, Carbopol, and poloxamer are utilised as gelling polymers, this approach is typically employed.
2. **Hot Method:** The hot method is usually utilized when pectin or gellan gum are employed as a gelling polymer. During the procedure, the gellan gum chains progressively dissolve in water at high temperatures and adopt a random-coil configuration with high segmental mobility. When this solution cools while being surrounded by ions like K+ or Ca2+, it progressively begins to gel. The demethoxylation of pectin, which aids in its solubilization, also requires high temperatures.[1,14]

Evaluation parameters of nasal In-Situ Gels

1. **Texture analysis:** To assess the cohesion, stiffness, and consistency of the gel formulation, texture analysis is used. It is used to gauge how easily the gel can be syringed out for use in vivo.[4]
2. **Measurement of Gelation Time:** A test tube containing 2 ml of the formulation is placed in an oven at 37° C. In-situ gel's gelation at a particular time is investigated. [4]
3. **Gel strength determination:** The modified Gel strength device was used for this test. In a 100 ml measuring cylinder, gel was placed. Simulated nasal fluid was added to induce gelation. The

tool for measuring gel strength was placed on top of the gel. The time (in seconds) needed to lower the device (a 35g piston) 5 centimetres through the gel was used to gauge its strength.[4]

4. **In vitro drug release studies:** The drug release investigations were performed using a plastic dialysis cell which is made of a receptor and a donor compartment. A cellulose-based membrane was used to separate the two compartments. The donor compartment is filled with the formulation. At predetermined times, a specified volume of the receptor solution can be withdrawn and replaced with new media. Using a particular analytical technique, the drug release from this receptor solution is examined.[4]
5. **Drug content determination:** After manually shaking the formulation-containing vials for two to three minutes, 100 µL of the preparation was transferred to 25 ml volumetric flasks, and the remaining volume was filled with enough phosphate buffer pH 6.2. A UV-vis spectrophotometer was used to calculate the amount of drug.[4]
6. **Nasal Mucociliary Transport Time:** This test determines how long an insitu gel remains in the nasal cavity. Both the physiological saline solutions and the insitu gel formulations (5 mg/mL methylene blue) were made. A sodium thiopental (7 mg/mL) intramuscular injection was used to anaesthetize 5 rats. Using a micropipette, 10 µL of each sample was then injected into the rat's right nostril. The throats of the rats were cleaned using damp cotton swabs, and the time taken for the appearance of the blue dye's was noted.[4]
7. **Histopathological studies:** The isolated sheep nasal mucosa from the local slaughterhouse was used for histopathological examinations. The tissue that was treated in phosphate buffer (pH 6.2) and the tissue that was incubated in the Franz cell's diffusion chamber within situ gel formulations were compared. The tissue was treated conventionally and then embedded in paraffin after being preserved in 10% buffered formalin (pH 6.2). Hematoxylin and eosin staining was performed following the cutting of these paraffin sections. The optical microscope was used to analyze the slices to look for morphological changes in the tissue.[4]
8. **Ex vivo permeation study:** Fresh sheep nasal mucosa was procured from the local slaughterhouse and fitted as a flat sheet in a two-chamber diffusion cell that is kept at 37 ± 0.5°C, with the mucosal side towards the donor compartment. The receptor compartment was then filled with phosphate buffer pH 7.4. 2 mL formulation was applied to the donor compartment's mucosal surface after a 20-min preincubation period. Simulated nasal fluid was used to cause gelation. At each sampling, 0.5 ml of the sample was removed from the receiver compartment at a specified time and replaced with the same volume of phosphate buffer pH 7.4. Samples that are collected were analysed. The permeability coefficient "p," which is expressed in cm/h, was calculated using: [4]

$$P = \frac{dQ/dt}{C0\ A}$$

Where,

p - dQ/dt is flux or permeability rate (mg/h),

C0 - Initial concentration of drug in the donor compartment and

A - Effective surface area of the nasal mucosa.

9. **pH of gel:** After preparing the insitu gel pH of the formulation is immediately checked using a calibrated digital pH meter. In the case of nasal preparations, the pH should between 5.5 to 6.5 pH to

avoid irritation and improve patient tolerance and compatibility.[5]

10. **Gelling capacity:** By adding one drop of a freshly made formulation to a vial containing two millilitres of stimulated nasal fluid (SNF), and recording how long it takes for the gel to develop and dissolve in phosphate buffer 7.4 pH. This test is utilised to determine the appropriate polymer concentrations or gelling agents. [5]
11. **Viscosity and rheology:** A Brookfield viscometer is used to test the viscosity at body temperature (i.e., 37±0.5 °C) and room temperature (i.e., 25 °C). To know the thixotropic behaviour of the gel, rheology was examined. The in-situ gel preparations should exhibit pseudo-plasticity and Newtonian flow both before and after the gelation procedure. It should be 50–50,000 m Pas ('gel') and 5–1000 m Pas ('sol'). [5]
12. **Appearance and Clarity:** The gel should have a clear appearance. To check the clarity, the formulations were visually inspected for colour and the presence of dispersed particulates on a white and black backdrop.[5,13]
13. **Sol-gel transition temperature:** The thermoreversible polymers that make up in situ gelling systems are known to undergo a phase shift at the so-called sol-gel transition temperature. The solution of the formulation is stored in a sample tube at a particular temperature and heated at a predetermined rate. The temperature at which the sol transforms into a gel is known as the sol-gel transition temperature. This temperature is shown by a lack of movement of the meniscus when the tube is tilted. [13]
14. **In Vitro Mucoadhesive Strength:** Using a specialised chemical balance, the amount of force needed to remove the in-situ gel formulation from the two nasal mucosae was calculated. One of the nasal mucosae was placed on top of the clear glass surface on one side of the balance and secured with a rubber band, while the other was positioned at the bottom of the left pan inverted so that it faced the first mucosa. After that, a 50mg of the in-situ gel formulation was applied between the two nasal mucosae and left there for a few minutes. Weight was gradually added to the right pan until two mucosae separated from one another. Mucoadhesive strength is expressed in terms of force or stress detachment per cm square area of the mucosa used. It is given by the equation: [15]

$$\boldsymbol{Detachment\ stress} = \frac{\boldsymbol{m} * \boldsymbol{g}}{\boldsymbol{A}}$$

Where,

m- Weight in grams required to detach the two mucosae

g - Acceleration caused due to gravity

A - Exposed surface area of the mucosal tissue in cm^2

Conclusion

As an alternate route for the administration of medications and biomolecules that are prone to gastrointestinal degradation or have negative side effects when taken orally, nasal drug delivery is a rapidly developing discipline. The nasal route avoids bioavailability problems and has the benefit of direct nose-to-brain administration via a variety of pathways. Patient compliance, which in situ gels can provide, is directly related to the effectiveness of any dose form. A "reliable and non-invasive option for nose-to-brain delivery that can overcome several drawbacks associated with conventional dosage forms" is what in situ nasal gelling systems can be described as.

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