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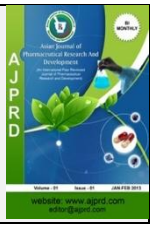

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Review Article

Pharmaceutical Considerations of Nasal In-Situ Gel As A Drug Delivery System

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ABSTRACT

In situ gelling drug delivery systems have gained enormous attention over the last decade. They are in a sol-state before administration, and they are capable of forming gels in response to different endogenous stimuli, such as temperature increase, pH change and the presence of ions. Such systems can be administered through different routes, to achieve local or systemic drug delivery and can also be successfully used as vehicles for drug-loaded nano- and microparticles. Natural, synthetic and/or semi-synthetic polymers with in situ gelling behaviour can be used alone, or in combination, for the preparation of such systems; the association with mucoadhesive polymers is highly desirable in order to further prolong the residence time at the site of action/absorption. Nasal drug delivery is a better alternative of oral and parental route due to high permeability of Nasal epithelium, rapid drug absorption, avoid Hepatic first pass metabolism, increased bioavailability of drug, minimized local and systemic side effects, Low dose required, Direct transport into systemic circulation and CNS is also possible (passing blood brain barrier), Improved patient compliance, Self-Medication is Possible, prevent Gastro intestinal tract Ulceration. Recently, it has been shown that many drugs have better bioavailability by nasal route than the oral route. Thus, this review focuses on nasal drug delivery, various aspects of nasal anatomy and physiology, nasal absorption mechanism, advantages & disadvantage composition of in situ gel, application and *In-situ* gels evaluations.

Keywords: Nasal *In-situ* Gel, Nasal formulation, Sustain drug delivery, Mucoadhesive Drug Delivery System Gelation, thermo-sensitive systems; ion-sensitive systems; pH-sensitive systems; Evaluation

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INTRODUCTION[1-9]

In the last decade, the development of in situ gelling drug delivery systems (especially nasal route) has gained an increasing attention in the scientific Research. The majority of these systems presents the uniqueness to be in a sol-state before administration and to undergo gelation into the cavity. As a consequence, they get a significance of easy administration, prolonged residence time, sustained drug release, reduction in administration frequency and an improvement in patient compliance and comfort. Among the reasons for the great success of these formulations, there is the fact that they can be administered through various routes to achieve a local or a systemic effect of the drug loaded.

There are many ways to trigger the sol–gel transition, such as temperature increase, pH change and presence of ions. Thermo-sensitive in situ gelling systems are in a sol-state at room temperature and are subjected to a sol–gel transition at temperature values close to the physiological one like 37 ^{0}C. Polymers, generally used for the manufacturing of such systems, are biocompatible, well tolerable and preferably mucoadhesive. Moreover, a pseudo plastic behavior is desirable: It could avoid a painful blink, since it guarantees

a reduction in the viscosity of the administered polymeric solution with increasing shear rate.

The nasal cavity has been emerged as an attractive route of multi-site targeting for the administration of a wide plethora of drugs, from small compounds to biological macromolecules, including peptides, proteins and vaccines.

The nasal route is the natural choice for the topical administration of drugs intended for the treatment of local disorders affecting the nose and the par nasal sinuses, such as allergic or infectious rhinitis, sinusitis, rhino-sinusitis and nasal epithelium lesions.

Moreover, the nasal route also proves beneficial for delivering drugs to the brain, avoiding the blood–brain barrier (BBB) that restricts the diffusional transport mechanisms of several therapeutic agents after oral or parenteral administration. The nose-to-brain delivery guarantees the direct and rapid transport of drugs from the nasal cavity to the central nervous system (CNS) through the olfactory neuroepithelium.

As intranasal route offers several advantages in terms of accessibility, efficacy, tolerability and patient compliance, the mucociliary clearance represents the physiological factor mainly involved in the reduction of the drug residence time in the nasal environment. Such a self-clearing mechanism is responsible for the rapid removal of the drug from the nasal cavity, thereby reducing the time needed for the drug to treat the nasal local diseases or to reach the systemic bloodstream or the CNS.

In an attempt to prevent the rapid drainage of drugs, when administered as simple aqueous solutions, and to prolong their residence time in the nasal cavity, a viscosity-enhancing approach (using polymers) has been proposed: nasal in situ gelling formulations seem to be a more effective alternative to nasal liquid ones.

Such formulations are easily administered as low viscosity polymeric solutions, ensuring an optimal nasal deposition, and turn into gels upon contact with the mucosa. The sol–gel transition can be induced by different physical or chemical stimuli, in particular temperature, pH and ionic strength: The in vivo formation of a polymeric network prolongs the contact time between the drug and the site of action/absorption and also guarantees a sustained release of pharmaceutical ingredients.

RECENT ADVANCMENT IN NASAL IN-SITU GELLING SYSTEM[10-16]

Dukovski and colleagues developed an in situ gelling formulation for corticosteroid delivery in the treatment of chronic rhino sinusitis with nasal polyps. In particular, dexamethasone loaded lipid/ALG nanoparticles were dispersed in a PEC solution that could be easily sprayed at the site of inflammation by an appropriate nasal delivery device; the ability of PEC to form gels upon contact with the Ca2+ ions present in the nasal mucosa is responsible for the sol–gel transition.

The use of thermo-reversible polymers for the preparation of nasal in situ gelling systems was also exploited by other authors, such as Atlanta's and Yener. In their work, P407 was used in combination with Carbopol® 974P NF, a bioadhesive polymer, in order to produce a thermo-sensitive gel for the prolonged release of Mometasone Furoate in the treatment of allergic rhinitis. The poloxamer concentration was optimized so that the sol–gel transition temperature (Tsol–gel) of the in situ gelling system developed was lower than the nasal physiological one. The increase in P407 concentration leads to a decrease of formulation Tsol–gel and, thus, to the rapid formation of a well-structured gel.

The poloxamer was used in combination with bioadhesive polymers, such as HPMC E4M and CS, in order to enhance the adhesion of the gel, loaded with dexamethasone 21-phosphate disodium salt, on the nasal mucosa. Nasal administration has aroused particular interest in the systemic drug delivery, since it improves patient compliance: Unlike parenteral administration, it is painless and allows self-medication.

Moreover, nasal delivery guarantees an accurate and consistent drug dosing, considering that nausea and vomiting are causes of gastric dysmotility and, thus, of significant alterations of drug intestinal absorption after oral administration.

In 2016, Sonje and Mahajan developed ondansetron hydrochloride OND-loaded in situ gelling nasal insert absorption across the mucosa. a selective 5-HT3 receptor antagonist used for the prevention of nausea and vomiting after radio- and chemotherapy or surgical operations.

A recent investigation conducted by Wavikar and co-workers has explored the potential of a nasal in situ gelling formulation incorporating rivastigmine (RV)-loaded NLCs in the systemic treatment of Alzheimer's disease. In order to ensure an optimal resistance to mucociliary clearance, GG and poloxamer (Lutrol 127), which are two polymers responsive to different stimuli (ions and temperature, respectively), were used in combination for the preparation of such system. Brain targeting potential of the formulation was assessed by in vivo pharmacokinetic and pharmacodynamic studies: RV concentration in the brain was 1.61 times more when the NLCs were intranasally administered in the in situ gelling system, as compared with intravenously administered ones Nose-to-brain delivery was

also exploited in the treatment of AIDS dementia complex, a CNS disorder that occurs when human immunodeficiency virus (HIV) enters in the brain tissues; in particular,

Ved and Kim prepared an in situ gelling system, based on the use of poloxamer as thermo-reversible agent, for the enhancement of intranasal zidovudine (ZVD) delivery to the brain. The in vivo absorption and brain distribution studies in rabbits revealed that the ZDV concentrations in both the cerebrospinal fluid (CSF) and the brain, achieved after intranasal administration of thermo-sensitive system, were approximately five times greater than those attained after intravenous injection.

ANATOMY AND PHYSISOILOGY OF NASAL CAVITY[17-20]

The Nasal cavity is divided into two halves by the nasal septum and extends posterior to the nasopharynx, while the most anterior part of the nasal cavity, the nasal vestibule, opens to the face through the nostril. Breathing and olfaction are the major function of human nose but it also functioned as filtration and humidifies inhaled air before reaching in lowest airway. Nasal cavity has mucus layer and hairs, those helpful in filtration of particles trapped in inhaled air. 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 via the septum. The volume of each cavity is approximately 7.5 mL, having a surface area around 75 cm2.

Three regions can be distinguished in each part:

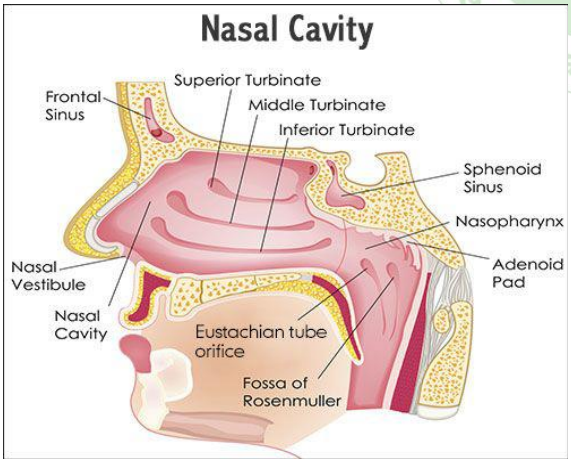

Figure 1: Anatomy of Nasal Cavity

The Respiratory region-The respiratory region is the largest having the highest degree of vascularity and is mainly responsible for systemic drug absorption. The respiratory epithelium is composed of four types of cells, namely, non ciliated and ciliated columnar cells, basal cells and goblet cells. These cells facilitate active transport processes such as the exchange of water and ions between cells and motility of cilia (where applicable). They may also serve to prevent drying of the mucosa by trapping moisture.

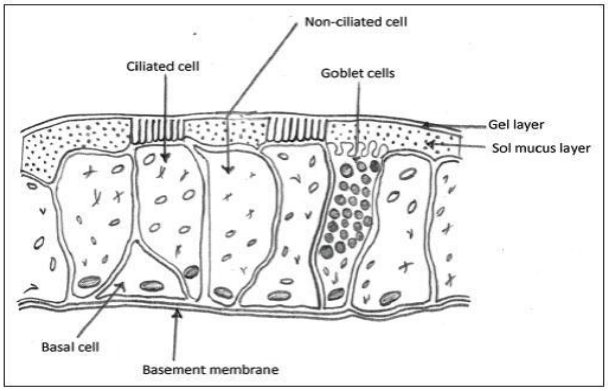

Figure 2: Cell types in nasal epithelium

2. Olfactory region- It is of about 10cm2 in surface area and it plays a vital role in transportation of drugs to the brain and the CSF. The olfactory region is located on the roof of the nasal cavities, just below the cribriform plate of the ethmoid bone, which separates the nasal cavities from the cranial Cavity. The olfactory tissue is often yellow in color, in contrast to the surrounding pink tissue. Humans have relatively simple noses, since the primary function is breathing, while other mammals have more complex noses better adapted for the function of olfaction. The olfactory epithelial layer predominantly contains three cell types: the olfactory neural cells, the subtentacular (also known as supporting) cells and the basal cells.

3. The Vestibular region- It is anterior part of nasal cavity. Surface area is 0.6 cm2. Nasal portion is covered by a stratified squamous keratinized epithelial with sebaceous gland. It is located at the opening of nasal passages and is responsible for filtering out the air borne particles. Drug absorption is very difficult in this region but it afforded high resistance against toxic environment. It is considered to be the least important of the three regions with regards to drug absorption.

Mechanism of Drug Absorption by Nasal Route

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 which 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.

The two mechanisms that include there:-

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 show poor bioavailability.

Second mechanism-It involves transport through a lipoidal route known as the transcellular process. It is responsible for the transport of lipophilic drugs that show a rate dependency on their lipophilicity. Drugs can also cross cell membranes by an active transport route via carrier-mediated means or transport through the opening of tight junctions. For example chitosan, a natural biopolymer from shell fish opens tight junctions between epithelial cells to facilitate drug transport.

ADVANTAGE & DISADVANTAGES IN-SITU GEL NASAL FORMULATION[21-24]

Advantage	Disadvantage
• Increased residence time of drug in nasal cavity. • Decreased frequency of drug administration. • Results in rapid absorption and onset of effect. • Avoids degradation of drug in gastrointestinal tract resulting from acidic or enzymatic degradation. • Low dose required. • Minimized local and systemic side effects. • Improved bioavailability of drug. • Direct transport into systemic circulation and CNS, is possible. • Offers lower risk of overdose of CNS acting drug • Improved patient compliance.	• Not well-known drug transport mechanism • Surface area are less as compare to GIT • Limited volume can be sprayed • Suitable for potent drug • Loss of dosage due to mechanical technical aspects • Mucosal damage • Nasal mucosal irritation • Irreversible damage of the cilia on the nasal mucosa.

APPROACHES FOR *IN-SITU* GELLING SYSTEM[25]

In-situ-forming gels are liquid which upon instillation undergo phase transition in the nasal cavity to form visco-elastic gel as a response to environmental change. The main approaches for *in-situ* gelling system can be classified as follows:

Physiological stimuli approach or stimuli-responsive *in-situ* gel systems

This approach is further sub classified as:

a. Temperature induced *in-situ* gelling system.

b. PH induced *in-situ* gelling systems.

c. Ion induced *in-situ* gelling systems

d. Induced photo polymerization gelation (UV Induced gelation)

i. Physical change in biomaterial

This approach can be further sub classified as:

a. Swelling mechanism.

b. Diffusion mechanism.

ii. Chemical reaction approach or chemically induced *in-situ* gelling system

This approach can be further sub classified as:

a. Ionic cross-linking.

b. Photo-polymerisation.

c. Enzymatic cross-linking.

VARIOUS POLYMERS AND OTHER AGENT USED TO PREPARE NASAL IN-SITU GEL

Polymer[26]

Polymers used in pH sensitive In-Situ gelling system

Polymers included in this class contain an acidic or a basic group that either accept or release protons when they are exposed to different environmental pH. Hence these are called pH sensitive polymers. Gelling of the solution is triggered by a change in pH. At pH 4.4 the formulation is a free-running solution which undergoes coagulation when the pH is raised by the nasal fluid to pH 7.4. Most of the pH sensitive polymers containing anionic group are based on PAA (Carbopol®, Carbomer) and its derivatives.

Carbopol[27]

Carbopol is a polyacrylicacid (PAA) polymer, which shows a sol to gel transition in aqueous solution as the pH is raised above its pKa of about 5.5. Carbopol (poly acrylic acid) is a well-known pH dependent polymer, which stays in solution form at acidic pH but forms a low viscosity gel at alkaline pH.Carbopol 934p and Carbopol 981 are mostly used as gelling agent.

Mechanism

The Mucoadhesive property is due to electrostatic interaction or hydrophobic interaction, hydrogen bonding. It is acidic molecule. When dispersed in water, carboxylic group of the molecule partially dissociate and form a coil. As it is pH sensitive polymer, increase in pH of solution result in swelling of polymer. The gelling effect is activated in two stages, neutralization of solution by addition of, sodium hydroxide or potassium hydroxide, triethanolamine.

Temperature sensitive polymers for In -situ gelling system[28-29]

There are some polymers which undergo large and unexpected physical and chemical changes in response to small external changes in their environmental conditions. Such polymers are called Stimuli-responsive polymers. They are also called as stimuli-sensitive, intelligent, smart or environmentally sensitive polymers.

Temperature sensitive polymers are most widely studied class of environmentally responsive polymer systems in drug delivery. This is because temperature is relatively easy to control and also easily applicable to both in vitro and in vivo. In this system, gelling of solution is triggered by alteration in temperature, thus sustaining the drug release. These hydrogels exists in liquid form at room temperature (20-25°C) and undergo gelation when comes in contact with body fluid (35-37°C).

Temperature sensitive polymers may be,

1. Positive thermo sensitive gels: This system has an upper critical solution temperature (UCST), such hydrogel contracts upon cooling UCST.
   E.g. Polymer networks of poly (acrylic acid)
2. Negative thermosensitive gels:This system have a lower critical solution temperature (LCST) and contract upon heating above the LCST.
   E.g. poly (N-isoprophylacrylamide)
3. Thermo reversible gels
   E.g. poloxamers/pluronics, tetronics

Poloxamer[30-32]

Poloxamer are water soluble tri-block copolymer consisting of two polyethylene oxide and polypropylene oxide core in an ABA configuration.

Among this family of copolymers, poloxamer 407 is a non-ionic surfactant with reversible gelation properties above a particular polymer concentration and a particular temperature. The gelation phenomenon is reversible and characterized by a sol-gel transition temperature (Tsol-gel). Below. The thermogelation is due to hydrophobic interactions between the poloxamer 407 copolymer chains.

Mechanism

By elevating the temperature, the poloxamer 407 copolymer chains start to aggregate into a micellar structure. At room temperature (25°C), it behaves as viscous liquid and is transformed to transparent gel when temperature increases (37°C). The formation of micelle structures is a result of the dehydration of the hydrophobic PPO repeat units and defines the initial step of gelation. At low temperature, it forms small micellar subunit in solution and increases in temperature results increase in viscosity leads to swelling to form large micellar cross-linked network.

Chitosan[33-34]

Chitosan, an amine-polysaccharide is a pH dependent, cationic polymer. Neutralization of chitosan aqueous solution to a pH exceeding 6.2 leads to the formation of a hydrated gel like precipitate. Adding poly salts, bearing a single anionic head, like glucose phosphate salts to chitosan aqueous solution can transform the cationic polysaccharides solution into thermally sensitive pH dependent gel.

Polymers used of ion sensitive in-situ gelling system[35]

In situ formation is based on chemical reactions, following chemical reactions cause gelation, undergoes in situ gelling in the presence of mono- and divalent cations , including Ca 2+ , Mg 2+ , K + and Na+. Alginic acid undergoes gelation in presence of divalent/polyvalent cations.

Gellan gum[36]

Gellan gum (commercially available as Gelrite TM or Kelcogel TM) is an anionic deacetylated, exocellular polysaccharide secreted by Pseudomonas elodea with a tetrasaccharide repeating unit of 1b-l-rhamnose, 1b D-glucuronic acid and 2b D-glucose.

Mechanism

By forming of double-helical junction zones followed by aggregation of the double-helical segments to form a 3-D network by complexation with cations and hydrogen bonding with water. Because human nasal mucosa is covered with approximately 0.1 ml mucus, which consists of sodium, potassium and calcium ions.

Sodium alginate[37]

Sodium alginate is a salt of alginic acid extracted from brown algae. It is a linear block polysaccharide consisting of two type monomers β-D-Mannuronic acid and α-L glucuronic acid residues joined by 1,4 glycosidic linkages. It is biodegradable and non-toxic and exhibit good Mucoadhesive property due to its carboxylic group.

Mechanism[38]

The monomers of alginate β-D-Mannuronic acid and α-L glucuronic acid are arranged as M-M block with altering sequence (M-G) block. Upon interaction of G block of polymer with calcium moieties resulting in the formation of homogenous gel. Mechanical strength and porosity of hydrogel depends on G: M ratio, type of cross linker used and concentration of alginate solution

Synthetic Polymers[39-40]

a) N-isopropylacrylamide copolymers

Poly (N-isopropyllacrylamide) is a non-biodegradable polymer with LCST, 32°C in water and cross linked gels of this material collapse around this temperature.

b) PEG/PLGA Block copolymers

A novel concept, which combines thermo gelation, biodegradability, and no toxicity, has been proposed for an injectible gel system with better safety and longer gel duration.

Pectin[41]

Pectins are a family of polysaccharides. Low methoxypectins readily form gels in aqueous solution in the presence of free calcium ions, which crosslink the galacturonic acid chains. Although the gelation of pectin will occur in the presence of H+ ions, a source of divalent ions, generally calcium ions is required to produce the gels that are suitable as vehicles for drug delivery.

Xanthan gum[42]

Xanthan gum is a high molecular weight extra cellular polysaccharide produced by the fermentation of the gram-negative bacterium Xanthomonas campestris. The primary structure of this naturally produced cellulose derivative contains a cellulosic backbone (β- D-glucoseresidues) and a trisaccharide side chain of β-D-mannose-β-D-glucuronicacid- α- D - mannose attached with alternate glucose residues of the main chain. The anionic character of this polymer is due to the presence of both glucuronic acid and pyruvic acid groups in the side chain.

Induced photo polymerization gelation (UV Induced gelation)[43]

Photo polymerization is commonly used for in situ formation of biomaterials. A solution of monomers or reactive micromere and initiator can be injected into a tissue site and application of electromagnetic radiation used to form gel. The photo reaction provides rapid polymerization rate at physiological temperature. The photo polymerization systems when introduced to the desired site via injection get photo cured in situ with the help of fiber optic cables and then release the drug for prolonged period of time.

Other Agent Used In Nasal In-Situ Gel Composition[17]

ii. Permeation enhancers:

Absorption enhancers increase transitorily the permeability characteristics of physiological membranes and are used to facilitate drug penetration through the skin, and different epithelia (buccal, nasal, intestinal, and rectal). The use of absorption promoters is thought to be helpful in the formulation of nasal preparations to increase therapeutic action of a drug or achieve an equivalent effect with a lower concentration of the active ingredients.

iii. Preservative:

Similar to ocular formulations, nasal formulations are multidose dosage forms and therefore require the addition of a preservative.

The preservatives used for this purpose include:

- Chlorobutanol (0.5% w/w)
- Parabens (0.2% w/w total)
- Benzalkonium chloride (0.002–0.02% w/v)
- Thimerosal (0.002–0.005% w/v),

iv. Buffers:

Buffers are compounds that resist changes in pH upon the addition of limited amounts of acids or bases. Buffer systems are usually composed of a weak acid or base and its conjugate salt. Nasal formulations are usually buffered within the pH range of 5.5–6.5 to preserve nasal function. For this purpose buffers (e.g. citrate, phosphate) are included in these formulations. pH of the formulation affects the ionisation of therapeutic agents and this will, in turn, affect the rate of systemic absorption of the therapeutic agent following nasal absorption.

v. Vehicle:

Nasal formulations are aqueous and therefore use purified water as the vehicle. Non-aqueous solvents are not used to formulate nasal dosage forms as these may interfere with ciliary function. Small concentrations of co-solvents (e.g. glycerol, polyethylene glycol, propylene glycol) may be employed to enhance the solubility of the therapeutic agent in the formulation. In addition glycerol acts as humectants and may therefore reduce or minimise irritation to the nasal mucosa.

vi. Tonicity:

Tonicity of the formulation to maintain ciliary function nasal formulations are formulated to be isotonic. The approach taken to achieve this is identical to that described

for parenteral formulations, i.e. the inclusion of a suitable salt (e.g. sodium chloride).

vii. viscosity-modifying agents:

As with other formulation types, control of the viscosity of nasal formulations is important as it directly affects both the ease of administration to, and the retention of, the formulation on the nasal mucosa. The viscosity of nasal formulations may be easily modified by the inclusion of a suitable hydrophilic polymer, e.g. methylcellulose, hydroxyethylcellulose, sodium carboxymethylcellulose, poly (acrylic acid). The viscosity of the formulation is usually modified to be similar to that of nasal mucus (to preserve nasal ciliary function).

viii. Antioxidants

These are included to enhance the chemical stability of therapeutic agents that are prone to oxidative degradation e.g. sodium metabisulphite, sodium sulphite.

EVALUATIONS OF FORMULATION

Clarity [44]

The clarity of formulated solutions can be determined by visual inspection under black and White background.

Texture Analysis [45]

The firmness, consistency and cohesiveness of formulation are assessed using texture analyser which mainly indicates the syringeability of the formulation can be easily administered *in-vivo*.

pH of the Gel [45]

For determining the pH of the formulation of nasal *in-situ* gel, taken 1 ml quantity of each Formulation transferred into a different beaker and diluted it with distilled water up to 25 ml and then pH of each formulation was determined by using pH meter.

Drug Content[45]

First 1 ml of formulation was taken in 10 ml volumetric flask. And then it was diluted with 10 ml of distilled water then volume adjusted to 10 ml, 1 ml from this solution again diluted with distilled water up to 10 ml after this absorbance of prepared solution was measured at particular wavelength of the drug by using U.V visible spectrophotometer.

Viscosity Measurement [17,45]

Viscosity of nasal *in situ* gel was measured by using (cone and plate viscometer) programmable Brookfield viscometer The viscosity of nasal *in situ* gel were recorded at various temperature from 4°c to 40 °c respectively against increasing the shear rate.

Gelling Temperature [45-46]

This test is especially for the thermosensitive *in situ* gel. In this 2 ml *in-situ* gel transferred to test tube and placed into water bath then the temperature of water bath increased slowly and constantly. Gel was allowed to equilibrate for 5 minute at each setting, and then formulation was examined for gelation. When the meniscus would no longer move upon tilting to 90° angle, this is known as a gelation temperature.

Gel Strength [17,45]

This parameter can be evaluated using a Rheometer. Depending on the mechanism of the gelling of gelling agent used, a specified amount of gel is prepared in a beaker from the sol form. This gel containing beaker is raised at a certain rate so pushing a probe slowly through the gel. The changes in the load on the probe can be measured as a function of depth of immersion of the probe below the gel surface.

Determination of Mucoadhesive Strength:[17,47]

Mucoadhesive strength is known as the force to detach the *in situ* gel formulation from nasal mucosal tissue, for determining the mucoadhesive strength we use modified special chemical balance .A small section of nasal mucosa of goat was cut & tied or fixed on 2 glass vial with the help of rubber band or thread and stored it at 37°c ±2°c for 10 minute and then50mg of gel was placed on first vial and it placed below the height adjustable balance, while on another hand second vial was fixed in inverted position to the underside of the same balance after this height both vial were adjusted and come in intimate contact for 5 minute to ensure the contact between nasal mucosal tissue and the *in situ* gel formulation. Then weight was put off on the other side of balance, until vials got detached, it expressed as the strength or stress in dyne/cm².

Stress is calculated by the formula: Detachment Stress (dyne/cm²) = M × G ÷ A Where M = wt required for detachment of two vials in gm G = acceleration due to gravity A = Area of tissue exposed.

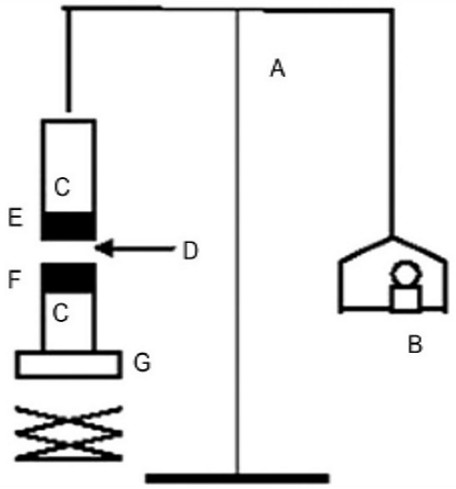

Figure.3: Modified Balance, B Weights, C Glass Vial, E, F Membrane, G Height Adjustable Pan**.**

In vitro diffusion study of in situ gel:

Franz having capacity 2.4 diameter and 15 ml diffusion cell was used for in vitro diffusion study of in situ gel. Dialysis (.22µm pore size) or cellophane membrane (12000-18000 mol wt) with diffusion area .8cm² used.60 ml of phosphate buffer (6.4-6.6pH) was prepared and membrane was soaked with phosphate buffer (6.4- 6.6 pH), after this temperature was maintained at 37°C±0.5°C, after this phosphate buffer placed into the acceptor chamber and gel containing drug equivalent to 10mg was placed in donor chamber, at predetermined time point, 1ml sample was withdrawn from acceptor chamber and then replaced the sample volume with equal amount of phosphate buffer after each sampling process, for a period of 300 minute, after each sampling, the samples were suitably diluted and measured spectrophotometrically at specific wavelength of drug. The concentration of drug was determined with the help of previous calibration curve.

***In vitro* Permeation Study of *In situ* Gel:**

To check permeation of drug and capacity of permeation enhancer which was added in formulation. Fresh nasal tissue section of goat obtains from slaughter house. Tissue was inserted in the diffusion cell. Gel containing drug equivalent to 10 mg was placed in donor chamber, at predetermined time point, 1ml sample was withdrawn from acceptor chamber and replacing the sampled volume with same amount of phosphate buffer, for a period of 300minute, after each sampling, the sample were suitably diluted and measured spectrophotometrically at specific wavelength of drug. **B. Permeability coefficient calculated from the slope of the graph:** P = Slope × Vd ÷ s Vd = volume of the donor solution S = surface area of tissue P = permeability coefficient. D.S.C (Differential Scanning Calorimetry), X Ray Diffraction and FTIT (Fourier Transform Infra –Red Spectroscopy) Studies: used for drug and polymer interaction, compatibility and to check matrix formation.

Histopathological Studies

Two mucosa tissue pieces (3 cm2) were mounted on in vitro diffusion cells. One mucosa was used as control (0.6 ml water) and the other was processed with 0.6 ml of optimized organogel (conditions similar to in vitro diffusion). The mucosa tissues were fixed in 10% neutral carbonate formalin (24 hours), and the vertical sections were dehydrated using graded solutions of ethanol. The subdivided tissues were stained with haematoxylin and eosin. The sections under microscope were photographed at original magnification ×100. The microscopic observations indicate that the organogel has no significant effect on the microscopic structure of the mucosa. The surface epithelium lining and the granular cellular structure of the nasal mucosa were totally intact. No major changes in the ultra-structure of mucosa morphology could be seen and the epithelial cells appeared mostly unchanged.

CONCLUSION

The present article is focus on the studying all the parameters/element of *in-situ* gelling system. Sustained and prolonged release of the drug, good stability and biocompatibility, patient compliance characteristics make the in situ gel dosage forms very reliable *in- situ* gel has some of the merits over the injectable administration and oral route are non-invasiveness and quick onset of action. Used of biodegradable, water soluble, thermo sensitive, pH sensitive polymer for the nasal *in situ* gel formulations can make them more acceptable and excellent drug delivery system. Nasal *in situ* gel enhanced the nasal residence time due to its viscosity an mucoadhesive strength. For optimum formulation can be achieved with better rheological properties, gelation time, gelation temperature, pH, mucoadhesive strength, and *in vitro* release and permeation studies.

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