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Formulation Development and Evaluation of Thermo-Responsive BIOGELS: Nose to Brain Approach

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ABSTRACT:

With an increase in nasal residence time, thermoreversible biogels can be useful as drug delivery methods. The purpose of this research was to develop a thermoreversible method for intranasal administration of doxepin using chitosan and glycerophosphate. To create the formulations, chitosan, glycerophosphate, polyethylene glycol, and the antidepressant doxepin hydrochloride were mixed in the appropriate concentrations. The gelling properties, rheology, mucoadhesion, in vitro release, and ex vivo penetration through sheep nasal mucosa of both systems were tested. Swiss albino mice were used for the forced swim test to determine the effectiveness in vivo. Mice that were repeatedly exposed to the formulation had nasal tissues examined histologically to see how the exposure affected them. All three formulations displayed thixotropy and gelled quickly at 37 degrees Celsius, then reverted to a sol upon cooling. Polyethylene glycol was added to make the formula isotonic and reduce the amount of glycerophosphate needed for gelation. The measured pharmacodynamic parameter suggested the formulation was effective in treated groups, and histopathological reports from those groups showed no substantial local toxicity. The biogels show promise as a platform for developing nasal medication delivery devices.

INTRODUCTION

The administration of drugs via the nasal route has been employed for the management of localized ailments such as nasal congestion, allergies, and infections. Furthermore, it has also been utilized for systemic drug delivery. The nasal mucosa presents several advantages as a viable target tissue for drug administration. These advantages include the early commencement of drug action, plasma drug profiles that closely resemble intravenous infusions, the potential for delivering drugs to the central nervous system, and the ability to avoid first-pass metabolism [1, 2].

Various strategies have been employed to address

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these limitations and boost the nasal bioavailability of pharmaceuticals. These approaches include the utilization of bioadhesives, permeation enhancers, in situ gelling systems, microspheres, and nanoparticles, among other techniques [3]. In situ gelling systems possess a distinct benefit due to their ability to transition from a liquid state at room temperature to a gel state at body temperature. This characteristic facilitates convenient administration and enables the gel to remain in the desired location for an extended period of time. Consequently, these systems have the potential to maintain the release of drugs over an extended duration. Several writers have reported on the use of thermoreversibly gelling devices for the intranasal administration of medicines [4, 5].

The pathway from the nose to the brain include the transportation of medicines through the olfactory system. The olfactory pathway is located inferior to the cribriform plate of the ethmoid bone, which serves as a partition between the cranial cavity and the nasal cavity. The olfactory neurons traverse the cribiform plate, which is encased by the arachnoid membrane housing subarachnoid cerebrospinal fluid (CSF) between the nerve and the membrane. The termination occurs at the olfactory sensory terminals, which extend into the olfactory mucosa. Consequently, the medicines have the ability to penetrate the brain parenchyma by means of CSF [6-7].

This study focuses on the formulation and evaluation of a new thermosensitive gel containing doxepin, which is based on chitosan-BG, for intranasal delivery. The produced systems underwent evaluation for many criteria, including gelling characteristics, rheological characteristics, in vitro drug release, and ex vivo penetration over sheep nasal mucosa. The efficacy of nasally delivered doxepin was assessed in vivo in mice, and the nasal mucosal irritation was investigated using histology.

MATERIALS AND METHODS:

The chitosan used in this study was obtained from the Central Institute of Fisheries Technology. β-glycerophosphate (BG) was procured from Central Drug House, while polyethylene glycol 4000 was purchased from S.D. Fine Chem. Doxepin hydrochloride was generously provided as a gift sample by Torrent Pharma Ltd. The porcine mucin used in this study was acquired from Sigma-Aldrich. The remaining chemicals and reagents employed in the investigation were of analytical grade.

2.1. Formulation of Gels

Thermoreversibly gelling systems were generated by combining components by a straightforward process under appropriate conditions. A solution of chitosan in 0.1 N hydrochloric acid (HCl) and an aqueous solution of BG were individually produced and subsequently chilled to a temperature of 4 degrees Celsius. Subsequently, a gradual addition of 1 mL of BG into the chitosan solution was performed with excessive agitation. Two distinct gelling systems were formulated: one comprising chitosan and BG at a greater concentration of BG, and another comprising chitosan, BG, and PEG 4000 as an additional component [8, 9].

Gels Characterisation

Gelation Time, Gelation Temperature, and pH

The gelling temperature of Formulation 1 (For. 1) and Formulation 2 (For. 2) and formulation 3 (for. 3) was determined using a process including the immersion of the sols in a water bath. The temperature was then gradually increased from 15∘C to 40∘C at a rate of 0.5∘C per minute. The temperature was held constant for a duration of 10 minutes at four different levels: 15∘C, 25∘C, 37∘C, and 40∘C. The tubes were periodically inverted until the movement of the meniscus ceased upon tilting of the tube. The measurement of gelation time involved determining the duration needed for the gel to cease flowing when the solutions were immersed in a thermostatic water bath set at a temperature of 37∘C. The pH values of all the solutions were measured at ambient temperature utilizing a calibrated pH meter [9, 10].

Viscosity of prepared gel

The rheological properties of all three Formulation 1, 2 and Formulation 3 were assessed using a Brookfield cone and plate viscometer. The viscosities of the samples were determined in both the sol and gel states by maintaining the samples at temperatures of 25∘C and 37∘C, respectively. The samples underwent shearing at rotational speeds ranging from 150 to 450 revolutions per minute (rpm) using spindle number 1. The rheological properties were determined by analyzing the relationship between rotational speed (RPM) and viscosity, as depicted in the graphs [10, 11].

In Vitro Release study

The gelled formulations were subjected to in vitro drug release investigations utilizing a Franz diffusion cell, with the experiments being conducted in triplicate. A parchment membrane was positioned between the donor and receptor chambers of the cells, with a contact area measuring 3.14 cm2. In the donor chamber, a gel weighing 2 g and containing 10 mg of the medication was introduced. The receptor phase, consisting of phosphate buffered saline at a pH of 6.4,

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was maintained at a temperature of 37∘C and subjected to continuous stirring using a magnetic stir bar for the duration of the experiment. Samples of 1 mL were removed from the receptor phase at predefined time points and subsequently replenished with PBS at a pH of 6.4. The collected samples were subjected to filtration [11, 12].

Mucoadhesion Property

The mucoadhesive force was assessed using a modified two-pan balance in both the sol and gel stages. Teflon blocks were affixed to the upper and lower sections of the left-hand side of the balance, while the right-hand side had a container designed for storing water. Mucin films were fabricated on coverslips by depositing 20 μL of a 3%w/v solution of porcine mucin in simulated nasal secretion onto a completely level surface, followed by allowing the films to undergo air drying. During the measuring process, the films were subjected to hydration for a duration of one minute using a small amount of simulated nasal discharge. The samples were subjected to a temperature of 25∘C for stabilization, and subsequently underwent gelation at 37∘C before being subjected to testing. The receptacle positioned in the right pan was filled with water at a flow rate of 5 mL/min using a peristaltic pump. The weight of water in grams necessary for the separation of the two surfaces was measured, and subsequently, the mucoadhesive force was determined [13, 14].

$F = W \times g$

In the given equation, the variable F represents the force of mucoadhesion, measured in dynes per square centimeter (dynes/cm2). The variable W denotes the minimal weight necessary to disrupt the mucoadhesive bond. Lastly, g represents the acceleration due to gravity, measured in centimeters per second squared (cm/s2). The data underwent a one-way analysis of variance (ANOVA).

Ciliary Function Evaluation

The determination of mucociliary transport time was conducted ex vivo in order to serve as an indicator of mucociliary function. The objective of this study was to evaluate the effect of different formulations and controls on the movement of opium poppy seed along the frog palate, with a focus on measuring the mucociliary transport time. The assessment protocol for evaluating ciliary activity in frogs received approval from the Institutional Animal Ethical Committee. The experimental procedure was conducted in compliance with the parameters set forth by the CPCSEA. The experimental procedure involved the pithing of frogs by flexing the head in a forward direction and subsequently putting a needle into the brain, followed by its insertion into the spinal cord. The disarticulation of the jaw and subsequent removal of the top portion of the head involved the use of scissors to cut from the junction of the posterior pharynx and esophagus to the skin of the back [15, 16].

***Ex Vivo* Permeation Study**

In order to conduct this study, nasal tissue samples were meticulously extracted from the nasal cavity of sheep at the Deonar Abattoir in Mumbai, with proper authorization obtained from the relevant authorities. The nasal mucosa was gently dissected from the septum, and the surrounding connective tissue and adherent cartilaginous tissue were meticulously excised using forceps and scissors, ensuring the preservation of the nasal mucosa without any damage or abrasion. The mucosa that had been separated was stored in phosphate buffered saline at a pH of 6.4 throughout transportation and was utilized within a time frame of 4 hours following the slaughter of the animals. The specimens were individually positioned on Franz-type diffusion cells and securely fastened between the donor and receptor compartments. The receptor phase consisted of 17 millilitres of phosphate-buffered saline (PBS) with a pH of 6.4, which was maintained at a temperature of 37 degrees Celsius [17-19].

***In-Vivo* Activity study**

The Institutional Animal Ethical Committee (IAEC), under the designation No. 242, granted approval for the animal testing methodology. The experimental procedure adhered to the requirements set forth by the CPCSEA for animal care and management. The formulations' effectiveness in vivo was assessed in Swiss albino mice through the evaluation of immobility duration and activity counts using a forced swim apparatus. The mice, regardless of their sex, were allocated into four groups of similar size using randomization [20, 21].

Histopathological Study

The nasal tissues of mice were subjected to histological examination in order to assess the potential harm or irritation caused by the formulations during in vivo efficacy testing. Following the conclusion of the efficacy investigations, a single Swiss albino mouse was randomly chosen from each group for the purpose of histological examination. The animals that were chosen underwent an additional daily dosage for two consecutive days, resulting in a cumulative exposure period of 15 days. The bones underwent decalcification after a 10-day treatment

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with 5% formic acid. Following a period of 10 days, the nasal tissues were isolated, subjected to a thorough water wash, and subsequently prepared for histological examination using varying concentrations of alcohol, xylene, and paraffin [22-24].

RESULTS AND DISCUSSION:

Gels Formulation

Chitosan exhibits high solubility in weakly acidic solutions with a pH below 6.0, while it tends to form precipitates when the pH exceeds this threshold. Chitosan exhibits water solubility at low pH due to the protonation of its amino groups. However, when the pH of chitosan solutions exceeds 6, the amino groups undergo deprotonation, causing the polymer to lose its charge and become insoluble. Nevertheless, the slow neutralization of the positively charged ammonium groups on chitosan chains can be achieved by the cautious addition of BG, a weak base, to a chitosan solution that is cold and acidic.

Table 1: Gelation characteristics study

Formulation	Polymer Conc.	BG Conc.	PEG Conc.	Temp.	Gelation (Time)
For - 1	**2**	05	1	36.5	08
For - 2	**4**	10	1	36.5	08
For - 3	**5**	15	1	36.5	08

Characteristics of gels

Gelation Time, Gelation Temperature, and pH

The critical concentration of BG necessary for chitosan to maintain solubility at higher pH levels and exhibit thermosensitivity is dependent on the degree of deacetylation and the molecular weight of chitosan. Initial investigations conducted in our laboratory have indicated that a minimum concentration of 8% of beta-glucan (BG) is necessary to confer thermoreversible qualities upon a 2% weight/volume (w/v) solution of chitosan. The gelation properties and pH of a gel containing 10% BG were observed to be optimal. Nevertheless, the high concentration of blood glucose leads to the formation of a hypertonic solution. Given the importance of isotonicity in formulations administered nasally, the use of PEG 4000 as an addition was investigated to aid in the production of a thermally gelling chitosan solution with a reduced concentration of BG. The solutions containing polyethylene glycol did not exhibit gelation immediately after being prepared, but underwent gelation at physiological temperature after being cured for a duration of 2 hours through storage at a temperature of 4 degrees Celsius [25, 26].

Viscosity Study

The rheological analysis revealed that the formulations exhibited shear thinning behaviour at both 25∘C and 37∘C. The systems also displayed a slight degree of thixotropy. Furthermore, across the full range of shear in which the experiments were conducted, it was seen that the viscosity of for. 3 gels consistently exceeded that of for. 1 and 2 gels at both temperatures.

***In-Vitro* Release study**

In the course of the release studies, it was observed that the gels exhibited no signs of degradation or disintegration on the surface of the parchment membrane after the 8-hour study period. All formulations exhibited an initial burst release of 20-25% of the contained medication, particularly within the first hour. The quick release phenomenon observed in this study may be due to the presence of unentrapped drug molecules that are scattered within the gel's tunnel network during the gelation process and then diffuse out rapidly. Subsequently, the medication that was encapsulated within the hydrogel exhibited a progressive release profile from the four tested gels. Approximately 70% of the administered medication was observed to be released within an 8-hour time frame. The incorporation of polyethylene glycol resulted in a reduction in burst release and a deceleration in the drug release rate from the gel. At the conclusion of an 8-hour period, only approximately 50% of Dox was released from the two formulations of gels [26, 27].

Mucoadhesion study

Chitosan is a polymer known for its mucoadhesive properties, which can be linked to the electrostatic attraction between the positively charged D-glucosamine units present in chitosan and the negatively charged sialic acid and sulphate residues found in mucus. At a temperature of 25∘C, it was observed that all formulations exhibited mucoadhesive properties that were either equivalent to or greater than those of the chitosan solution. In the solid form, the chitosan chains exhibit a high degree of mobility, allowing for effective interaction with the mucin and so facilitating strong mucoadhesion. The inclusion of the medication did not yield a statistically significant impact on the mucoadhesive properties of the For. 1 formulations.

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Ex Vivo Permeation Study

The penetration of doxepin across excised sheep nasal mucosa from a saturated water solution was found to be satisfactory. The Papp values of doxepin provide insight into the appropriateness of the selected candidate for intranasal administration. The release and permeation patterns exhibited by For. 1 and 2gels and For. 3 gels demonstrate comparable trends. The Papp values for doxepin obtained from the formulation of 2 gels and the drug solution were analysed. The penetration rate of doxepin from a gel matrix is contingent upon the rate at which it is released from the matrix.

In Vivo Activity

The phenomenon of behavioural despair, also known as forced swimming, is rooted in the notion of quantifying the length of immobility that occurs when rodents are subjected to an unavoidable scenario. The observed behaviour is indicative of a state of despair, which can be mitigated by many therapeutic medications that have demonstrated efficacy in treating depression in humans. Most antidepressants commonly prescribed in clinical settings have been shown to reduce the length of immobility.

Histopathological Investigation

The nasal tissues in the control group, which received saline administration, exhibited no abnormalities, and there was no observed alteration in the nasal epithelium. The application of a pharmacological solution to nasal tissues appears to have caused notable harm to the nasal mucosal tissues, as shown by the presence of mild infiltration and glandular hyperplasia, as well as severe epithelial hyperplasia. The occurrence of sluffing, resulting from the administration of doxepin, was found to be diminished when the drug was supplied in the form of gels.

Table 2: Histopathological Investigation

Sr. No.	Features	For. 1	For. 2	For. 3	Pure Drug Solution
1.	Epithelial Hyperplasia	Mild	Moderate	Mild	Mild
2.	Inflammatory cells inflammation	Mild	Mild	Mild	Mild
3.	Congestion	-	-	-	-
4.	Sluffling of mucosal epithelium	-	-	-	Mild
5.	Glandular hyperplasia	Mild	Mild	Mild	Mild

Remarkably, notwithstanding the hypertonicity linked to this particular system, it was observed that the for. 1 formulation exhibited superior tolerability with less harm to epithelial tissues. The findings indicate that the medication demonstrates favorable nasal acceptability in mice when administered in the form of chitosan-based in situ gelling formulations.

CONCLUSION

The findings presented in this study demonstrate that chitosan/BG systems exhibit promising potential as intranasal in situ gelling thermosensitive formulations for the delivery of central nervous system acting drugs. This is attributed to the systems' optimal gelation properties, safety profile, and effectiveness. Furthermore, as a result of the prolonged impact of the formulations, there is potential to decrease the frequency of dosing.

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Conflict of Interest

None

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