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Design and assessment of in-situ nasal gel incorporated with nanostructured cubosomes for the targeted therapy of schizophrenia

Avinash TEKADE*, Mukesh RATNAPARKHI, Ashutosh SHEWALE, Rushikesh SHINDE, Gajanan KULKARNI, Parth PAWAR,

[1] Department of Pharmaceutics, Marathwada Mitra Mandal's College of Pharmacy, Pune-411033(M.S.), India.

* Corresponding Author. E-mail: avitekade@gmail.com (A.T.).

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ABSTRACT: This study aimed to pioneer an innovative treatment tactic for schizophrenia by harnessing the potential of haloperidol (HPD) loaded nanostructured cubosomes infused into an in-situ nasal gel. An Artocarpus heterophyllus L. (Jackfruit) mucilage (AH mucilage) was used as a novel mucoadhesive substance in intranasal formulation. The formulation strategy included high-pressure homogenization (HPH) employing poloxamer 407 functions as a surface-active agent, serving the role of a surfactant, while polyvinyl alcohol is employed as a stabilizing agent in the formulation. The resulting HPD cubosomal structure exhibited particle size ranging from 64 to 198 nm, facilitating efficient brain delivery through the nasal route. Among the prepared batches, H7 stood out notably with a particle size (PS) of 156 ± 7.46 nm, the polydispersity index (PDI) was determined to be 0.2667, indicating the degree of heterogeneity in the particle size distribution. Simultaneously, the zeta potential (ZP) was measured at -21.71 mV, signifying the electrostatic charge on the particles in the solution. Entrapment efficiency of 78.80 ± 0.76% and drug content of 84 ± 1.00% were achieved. Transmission electron micrographs vividly illustrated the cubosomal morphology. In ex-vivo investigations conducted on sheep nasal mucosa, the cubogel formulated with AH mucilage displayed superior nasal mucoadhesion and enhanced drug permeation in comparison to Carbopol P934, a synthetic mucoadhesive agent. In conclusion, the developed HPD cubogel, incorporating mucoadhesive mucilage from Artocarphus heterophyllus L., presents a hopeful and inventive strategy for the effective management of schizophrenia.

KEYWORDS: Schizophrenia; cubosomal in-situ gel; Haloperidol; *Artocarpus heterophyllu*s L. mucilage; nose to brain delivery.

1. INTRODUCTION

According to a World Health Organization report, 1 in 300 persons, or a total of around 24 million people, suffer from schizophrenia globally [1]. Typically emerging in early adulthood, schizophrenia significantly influences a patient's quality of life. This complex disorder manifests through a spectrum of symptoms, encompassing positive aspects such as hallucinations, delusions, disorganized thought, or speech, alongside negative facets like reduced motivation, social withdrawal, and flattened emotions. Additionally, cognitive challenges, including memory and attention deficits, further characterize this multifaceted condition. It has been linked to disturbances in various neurotransmitter systems. However, the dopaminergic system seems to be the most damaged neurotransmitter system in schizophrenic patients, with a malfunction in dopamine production that either rises or reduces [2]. As a result, antipsychotic medications that primarily target dopamine receptors are commonly used in treatments [3].

Haloperidol (HPD) is a common antipsychotic drug that blocks dopamine D2 receptors in the brain. It is only available in oral or injectable forms, which have low availability at the site of action and high side effects. The challenge with HPD lies in its necessity to traverse the blood-brain barrier, restricting its access to the brain. Furthermore, liver enzymes break down HPD, leading to its distribution to various body parts, potentially triggering complications like movement disorders, sedation, weight gain, prolactin alterations, and cardiac events. A substantial portion, up to 50–60%, of orally administered HPD undergoes hepatic metabolism, substantially limiting its bioavailability. The intranasal route is a better way to deliver HPD, a

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common antipsychotic drug, to the brain. This is because it is easy, painless, and bypasses the liver and the BBB, which increases the drug's effectiveness and decreases its side effects. Thus, the intranasal route allows HPD to reach the brain quickly and directly through the nerves in the nose, which is important for treating psychiatric emergencies. HPD possesses the coveted traits essential for central nervous system (CNS) targeting, including its diminutive molecular weight, remarkable potency necessitating a dosage below 20 mg, and sufficient lipophilicity, facilitating its smooth traversal through the blood-brain barrier (BBB). These distinctive features emphasize HPD's suitability for precise and effective CNS-focused interventions [4].

The aim of this investigation was to create and assess nasal gel containing cubosomes, specifically designed for the intranasal administration of HPD. This approach holds promise for addressing psychiatric emergencies while minimizing adverse effects linked to delivery to the intended target site and enhancing overall drug presence in the body. In this research, a comparative analysis was conducted, juxtaposing a newly introduced natural mucoadhesive agent (Artocarpus heterophyllus L. mucilage) against a synthetic counterpart, both integrated into the in-situ nasal gel. This deliberate choice aims to enhance CNS targeting, ultimately elevating HPD concentration within the brain through the intranasal route.

2. RESULTS and DISCUSSION

2.1. Characterization of HPD Cubosomal Dispersion

2.1.1. Assessment of PS, PDI and ZP

HPD cubosomal dispersion was evaluated for PS, PDI, and ZP to affirm compliance with a formulation prepared for intranasal neurodelivery. The observed values were found in the specified acceptable parameters for intranasal drug delivery, the size of cubosomes plays a crucial role in determining the permeation of drugs through the nasal cavity. Previous investigations underscore the importance of nanoparticulate drug delivery systems, indicating that effective CNS targeting is attainable when the particle size (PS) remains below the threshold of 100 nm [5]. In the olfactory and trigeminal transport routes the olfactory axon, with an average diameter spanning from 100 to 700 nm in humans [6], sets a parameter for effective drug transport. Therefore, to attain efficient drug delivery to the brain through the intranasal pathway, it is crucial to maintain an optimal particle size falling within the range of 100 to 300 nm [7, 8]. Recent studies propose that cubosomes exhibit a safe and efficient ability to bypass the BBB. It is proposed that cubosomes have the potential to be directly transported through the nasal cavity to the brain, potentially preventing or at least reducing opsonization. This ensures the secure conveyance of HPD to the brain. [9]. Notably, the PS (particle size) of the prepared HPD cubosomes falls within the optimal range of 64 nm to 198 nm, rendering them well-suited for effective transportation from the nasal cavity to the brain (Table 1). The impact of lipid concentration, specifically Glyceryl Monooleate (GMO), was markedly evident in influencing particle size. Notably, an increase in GMO concentration, while maintaining stabilizer and surfactant levels constant, led to a significant rise in particle size, exemplified by formulations H6 and H8. Conversely, the concentration of surfactant P407 exerted a substantial influence on cubosomal particle size. Elevating surfactant concentration, as observed in H3 and H11 batches, resulted in a reduction in particle size compared to batches H2 and H8, characterized by lower surfactant concentrations. Nevertheless, a higher surfactant concentration contributed to stable dispersion, maintaining a consistent particle size. It's noteworthy that an increase in lipid concentration correlated with an augmentation in particle size, while the role of surfactant exhibited an inverse effect, diminishing cubosomal particle size when used in excess [10].

In the context of drug delivery using carriers based on lipids like liposomes or cubosomes, a Polydispersity Index (PDI) of 0.3 or lower is considered acceptable, signifying a uniform population of phospholipid vesicles [11]. The observed PDI values for the formulated HPD cubosomes ranged from 0.2011 to 0.8354. Notably, batches H7 and H9 exhibited a PDI below 0.3, signifying the most favorable PDI value indicative of uniform particle size (PS) distribution in cubosomes.

Zeta potential, a critical parameter influencing nanocarrier stability, is closely tied to surface charge, reflecting the extent of repulsion between particles bearing similar charges in a dispersion [12, 13]. The HPD cubosomes displayed a negative zeta potential value (Table 1), for the selected batch which is -21.71 mV. This suggests that the formulated HPD cubosomes possess the potential for prolonged stability, further enhancing their suitability for sustained applications.

Table 1. PS, PDI, ZP, %EE and % DC of formulated batches

Formulation Batches	Particle Size (nm)	PDI	Zeta Potential (mV)	pH	% EE	% Drug Content
H1	114±4.24	0.3418	-29.348	6.1	68.46±0.98	70.07±1.21
H2	115± 4.88	0.5129	-19.182	6.3	86.15±0.89	89.40±1.01
H3	95±2.697	0.4799	-38.41	7.2	72.76±0.78	76.86±1.13
H4	146±0.75	0.3908	-17.66	6.3	86.52±0.81	90±1.06
H5	159±2.12	0.6748	-24.8	7.1	71.16±0.67	73.98±1.00
H6	198±3.53	0.8354	-24.89	6.0	66.47±0.92	69.03±1.12
H7	156±7.46	0.2667	-21.71	6.4	78.80±0.76	84±1.00
H8	177.4±3.418	0.4404	-15.856	6.8	63.81±0.63	66.88±0.98
H9	162±4.94	0.2011	-12.15	6.7	75.46±0.32	77±0.89
H10	117±3.53	0.6746	-23.326	6.2	68.13±0.87	70±0.85
H11	167±9.89	0.6564	-36.58	6.9	52.83±0.78	54.46±1.15
H12	149±4.94	0.5829	-16.2	7.5	84.66±0.76	88±1.23
H13	64±1.285	0.6003	-10.92	7.3	88.77±0.45	93.08±1.25

Each value in the table is represented as mean ± SD (n=3)

2.1.2. Assessment of %Entrapment Efficiency and Drug Content

Entrapment efficiencies (%) of all 13 batches were determined (n=3) and were found in the range of 52.83±0.23 to 88.77±0.89 %. The higher % EE might be due to the higher capacity of the lipid i.e. GMO to entrap the HPD within its core and due to avoidance of drug leaking [14, 15]. The drug content of HPD cubosomes was found in between 54.46±1.15 to 93.08±1.25 %. Table 1 shows the results of % EE and % DC for all 13 batches of HPD cubosomes.

2.1.3. TEM imaging of Optimized HPD Cubosomal Dispersion

The TEM images of the selected H7 batch (Figure **1**) revealed that the formulated cubosomes were in a cubic shape [16, 17]. They had irregular polyangular as well as cubic structures. The individual particle size observed in the TEM images was found in the range of 63 to 250 nm.

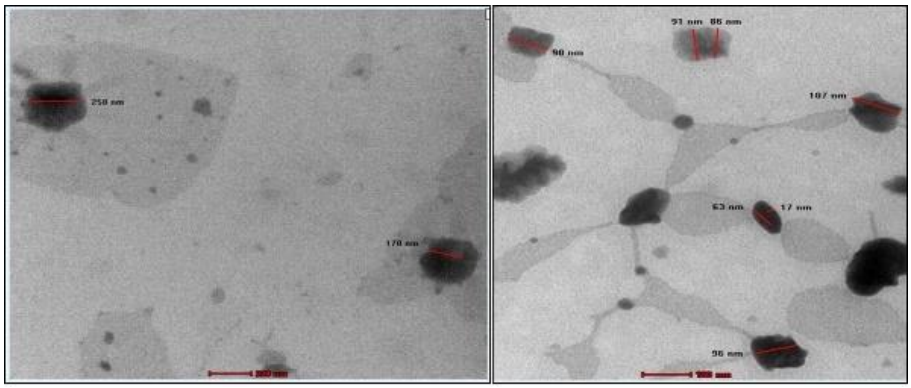

Figure 1. Transmission electron micrograph of HPD cubosomes

2.1.4. In-Vitro Permeation Study

This study of all HPD cubosomal dispersions was performed using a Franz diffusion cell setup. The experimentation involved employing a dialysis membrane submerged in a PBS (pH 6.4), as a diffusion medium. The release was found in the range of 48 to 91% for the prepared HPD cubosomal dispersions

(Figures **2** and **3**). For the selected batch (H7), the drug liberation from the lipid core (GMO) gradually increased from 5% at 30 min. to 72% at 360 min., indicating a slow and sustained diffusion process. The diffusion kinetics of the chosen HPD cubosomal dispersion (H7) revealed a progressive pattern, with 35.71% of the substance diffusing after 2 h. This diffusion rate notably increased to 72.141% by the conclusion of the 6 h assessment period (Figure **4**). Thus, it can be inferred that the release pattern of Haloperidol from the prepared cubosomal dispersion exhibited an initial burst release, followed by a sustained release that persisted over the entire 6 h experimental period. The observed rapid release initially is ascribed to the liberation of the adsorbed drug close to the surface of the cubic nanoparticles. This initial stage is defined by the prompt liberation of Haloperidol from the aqueous channels situated in the upper layers of the cubic structure. This process is facilitated by the hydrophilic coating afforded by polyvinyl alcohol (PVA) [17]. The subsequent gradual release aligns with the diffusion from the inner water channels, where Haloperidol is released at a slower pace. The sustained release is attributed to Haloperidol entrapped within the cubosomes, a result of the unique structure of these well-organized nanocarriers. Additionally, given that Glyceryl Monooleate (GMO) is an integral element of cubosomes, the gradual partitioning of HPD from the lipid-based matrix to the water-based matrix is plausible [17, 18]. In analyzing the release kinetics for the selected batch, the Higuchi model exhibited a noticeable pattern with the highest correlation coefficient (R^2 = 0.9695), closely followed by the zero-order model (R^2 = 0.9595). The Korsmeyer-Peppas model's coefficient (n) was determined to be 0.7572, indicative of anomalous (non-Fickian) diffusion. This behavior encompasses both drug diffusion and matrix dissolution, adding depth to the understanding of the intricate release mechanisms at play.

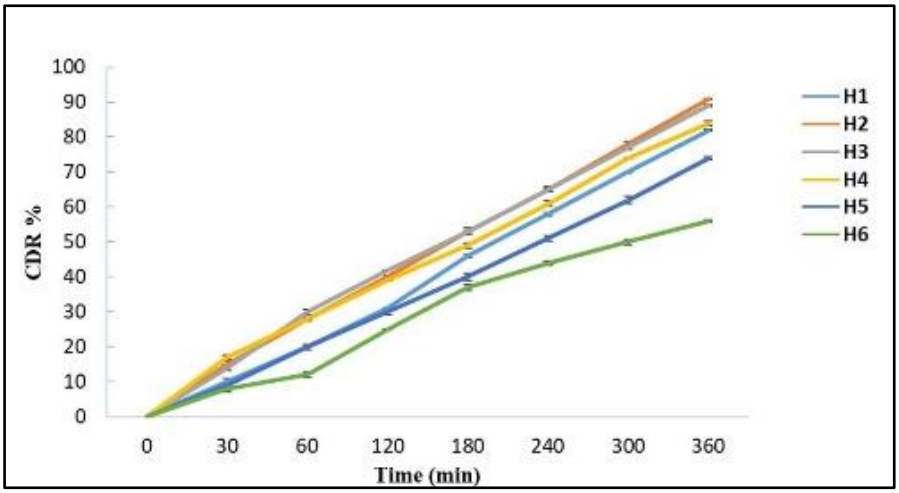

Figure 2. % CDR of HPD cubosomal dispersion from batches (H1-H6)

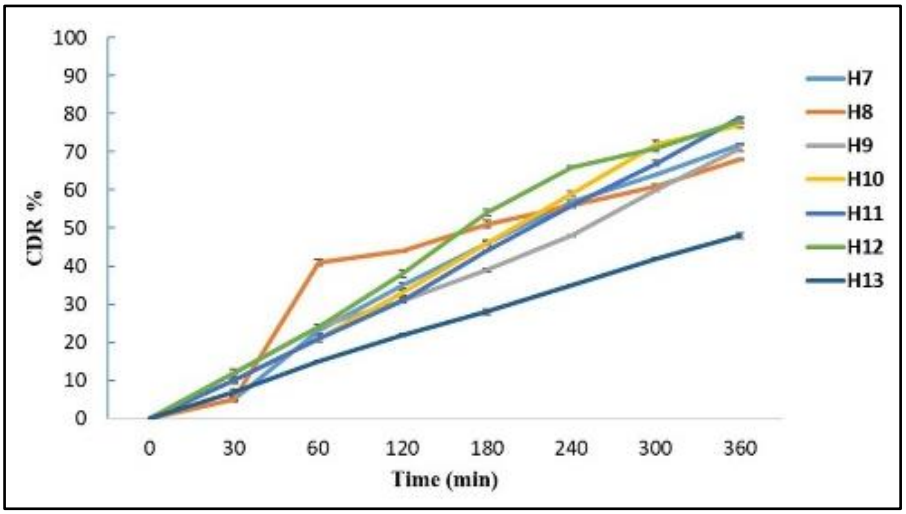

Figure 3. % CDR of HPD cubosomal dispersion from batches (H7-H13)

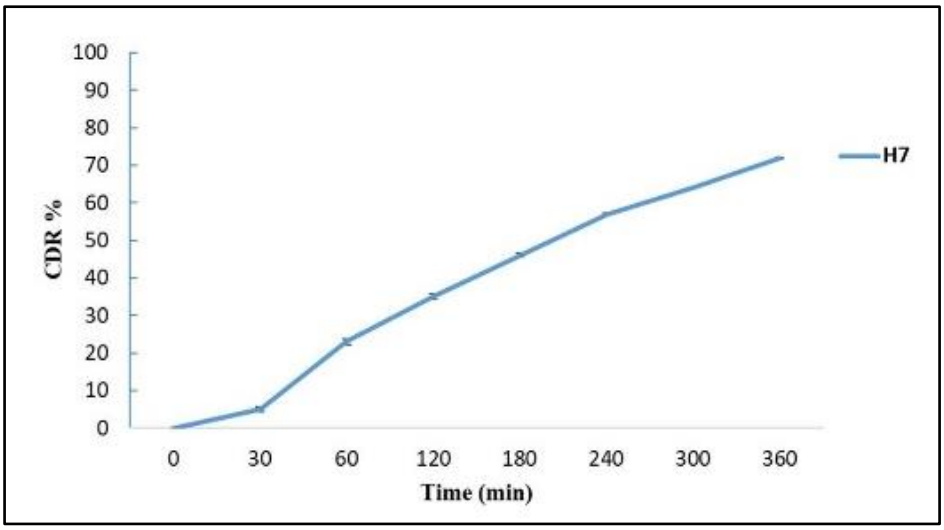

Figure 4. % CDR of the selected Batch H-7

2.2. Characterization of Nasal Cubosomal in-situ Gel of HPD

2.2.1. pH

Formulation batches showed a pH in the range of 6.0-7.5 which is suitable for the nasal mucosa because of the presence of lysozyme in nasal secretions, which functions to eliminate specific microbes in an acidic environment. In an alkaline pH, lysozyme loses its effectiveness, exposing the nasal tissue to the risk of microbial infections and potential irritation. Because it is an intranasal formulation, the appropriate pH of HPD cubogel is a very important factor in preventing irritation to the nasal mucosa.

2.2.2. Gelation Assessment

The optimization of mucoadhesive in-situ gel incorporating cubosomes demonstrated an exceptional degree of gelation, denoted as +++, indicating an instantaneous gel formation that persisted over an extended duration, characterized by a robust and long-lasting gel structure.

2.2.3. Assessment of Viscosity

The fluid thickness of the gel was determined using a Brookfield viscometer (Model- LV DV-E probe II, Brookfield Engineering, USA). The viscosity can be estimated using spindle no. 63 at 100 rpm. The readings for the gel formulations, one containing Carbopol P934 and the other incorporating AH mucilage, were found to be 577.1 cps and 1245 cps, respectively. These results highlight the unique rheological properties of each formulation.

2.2.4. Determination of Gel Strength

The robustness of the gel was influenced by the concentrations of the gelling and bioadhesive agents, namely Carbopol P934 and AH mucilage. It is imperative for a nasal gel formulation to exhibit an appropriate gel strength. This investigation illuminated that, at a temperature range of 33–34°C, the nasal formulation's gel strength improves with an increase in the gelling agent concentration. Also, the gel strength of the AH mucilage (93 sec) was found to be significantly higher than the carbopol P934 (55 sec).

2.2.5. Determination of Mucoadhesive Strength

The optimized HPD nasal in-situ cubogels (containing Carbopol P934 & AH mucilage) were subjected to a mucoadhesive study. The mucoadhesive strength stands as a critical parameter in cubosomal in-situ gelling formulations, playing a vital role in extending the gel remains in the nasal passages. Notably, the mucoadhesive force exhibited a proportional increase with rising mucilage concentration. This correlation underscores that a stronger mucoadhesive force results in decreased fluid drainage from the nasal cavity to the nasopharynx, highlighting its importance in enhancing the formulation's performance. The optimized HPD cubogel formulated using Carbopol P934 and AH mucilage showed mucoadhesive strength of 812 and 960 dyne/cm^2 respectively.

2.2.6. Ex-Vivo Study

The result of HPD permeation from the optimized HPD cubogel containing AH mucilage, and Carbopol P934 formulations is shown in Table **2**. The comparative assessment of the optimized HPD cubogel, incorporating AH mucilage and Carbopol P934, highlighted its efficacy in permeating Haloperidol (HPD) through the nasal membrane of sheep. The optimized HPD cubogel containing AH mucilage exhibited significantly enhanced drug permeation in both rate and extent when compared to its counterpart containing Carbopol P934. These noteworthy findings can be attributed to the presence of AH mucoadhesive mucilage, which, due to its superior adhesive properties, contributes to prolonged retention in the nasal area, consequently facilitating heightened permeation of HPD. This is in contrast with the synthetic mucoadhesive agent, indicating the potential superiority of natural mucilage in enhancing drug permeation through the nasal membrane.

Table 2. Ex-vivo Drug Permeation

Time (min)	CarbopoP934	AH mucilage
0	0	0
30	2.69±0.67	5.68±0.75
60	4.84±0.23	11.29±0.77
120	7.26±0.7	15.67±0.91
180	10.26±0.89	20.27±0.72
240	12.71±0.45	24.91±0.76
300	15.57±0.85	28.77±0.92
360	18.56±0.78	32.57±0.86

Each value in the table is represented as mean ± SD (n=3)

2.2.7. Nasal Histopathological Study

Microscopic analysis indicates that the optimized formulation does not negatively affect the mucosal microscopic structure, as illustrated in Figure **5**. No signs of cell necrosis or removal of the nasal mucosa epithelium were observed after applying the cubosomal in-situ gel and pH 6.4 buffer (used as the negative control). In stark contrast, damage to the nasal mucosa is evident in the positive control, specifically in the case of Isopropyl Alcohol (IPA). This observation underscores the formulation's biocompatibility, emphasizing its potential as a safe and non-deleterious nasal drug delivery system.

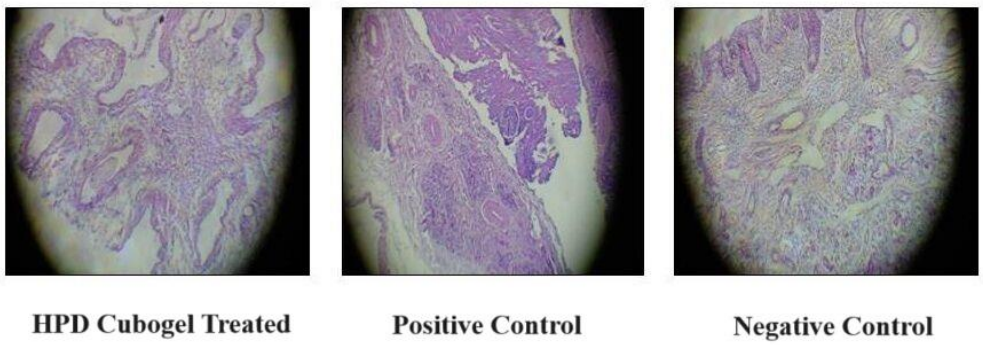

Figure 5. Effect of HPD cubogel on sheep nasal mucosa

2.2.8. Stability Study

A stability assessment was conducted on the chosen HPD cubosomal dispersion (H7) on 0,30,60, and 90 days at 4°C, with the observed changes meticulously documented in Table **3**. The PS and PDI increase with time due to the effect of GMO. However, the decreased value of ZP suggests that agglomeration might happen during this phase along with an increase in particle size as well as PDI.

Table 3. Stability study of HPD cubosomal dispersion

Period (Days)	Particle size (nm)	PDI	ZP (mV)
0	156	0.2667	-21.71
30	173.6	0.2933	-16.2
60	177.4	0.3077	-12.15
90	180.1	0.3857	-11.58

3. CONCLUSION

In this investigation, we formulated and assessed a nasal in-situ gel incorporating HPD-loaded cubosomes in combination with natural mucoadhesive agents. Transmission electron micrographs revealed typical irregular polyangular as well as cubic structures of formulated cubosomes. Ex-vivo study on sheep nasal mucosa revealed that HPD cubogel containing AH mucilage achieves higher permeation of HPD than the synthetic mucoadhesive agent that is Carbopol P934. Based on our investigation, it is deduced that the cubosomal in-situ formulation loaded with HPD presents a promising avenue for effective haloperidol delivery to the CNS via the intranasal route. Nevertheless, to validate and solidify the outcomes derived from the in vitro and ex vivo studies, an in vivo study utilizing a suitable animal model is imperative. This step will provide a comprehensive understanding of the formulation's performance in a biological system, thereby enhancing the translational potential of this innovative drug delivery strategy.

4. MATERIALS AND METHODS

4.1. Materials

The gift sample of Haloperidol was generously provided by Vamsi Labs Limited, situated in Solapur, India. Mohini Organics Pvt. Ltd. in Mumbai generously supplied Glyceryl Monooleate (GMO). Essential chemicals, including Poloxamer 407 (P407), Polyvinyl alcohol (PVA), sodium stearate, and sodium alginate, were procured from Loba Chemie, India. Kolliphor 188 and Carbopol P934 were thoughtfully contributed as gift samples by BASF, India. The Artocarphus heterophyllus L. (AH mucilage) was obtained from AH fruit, purchased from the local market in Pune.

4.2. Design and Optimization of Formulation Batches

The design and analysis were conducted using software i.e. Design Expert® (Version 8.0.7.1; M/s Stat-Ease Inc., Minneapolis, MN, USA) to scrutinize the effect of various formulation parameters on the attributes of HPD cubosomes. This investigation utilized a Box–Behnken Design (BBD) detailed in Table **4**, where GMO (mg), P407 (mg), and PVA (mg) served as the independent variables, with their actual values provided in Table **5**. The concentration of HPD was consistently maintained at 1 mg/ml across all formulations. The variables under examination are Particle Size (PS), Zeta Potential (ZP), and Entrapment Efficiency (EE). The optimization goal was to reduce size, enhance surface charge, and fine-tune entrapment, with the ultimate aim of creating a formulation with the highest desirability.

4.3. Isolation of Mucilage from Artocarpus Heterophyllus L. (Jackfruit)

Extraction of AH mucilage was carried out as per the earlier reported method [19, 20]. Initially, the fruits underwent a thorough washing process to eliminate any dirt. Subsequently, incisions were made, and the fruits were left overnight before being cut into pieces. After removing the seeds, the fruit pulp underwent crushing using a grinder. The ground pulp was then subjected to soaking in water for 5-6 h., followed by boiling for 30 min. and permitting it to stand for an additional hour to ensure the thorough release of mucilage into the aqueous medium. Mucilage extraction was accomplished using either a multi-layer muslin cloth or a vacuum pump to separate the marc from the solution. Ethanol, added to the marc in a volume three times that of the filtrate, was employed for mucilage precipitation. The obtained mucilage was isolated, subjected to drying in an oven at 35℃, collected, and subsequently stored in a desiccator for subsequent analysis and study.

4.4. Formulation of Cubosomal Dispersion

The melt emulsification method was used to prepare cubosomes with a minor alteration [21]. In this procedure, GMO and Poloxamer 407 were heated 5°C above melting point separately in a beaker using a heat-regulating water bath set at 70 °C. HPD (50mg) was dissolved in 5 ml of ethanol, and the volume was adjusted to 25 ml by adding distilled water. This resulting diluted mixture, with a concentration of 2 mg/ml of HPD, was introduced into the molten lipid, and mixed into the preheated aqueous Poloxamer 407 and Polyvinyl alcohol solution. Ethanol was included as a hydrotropic solvent to facilitate mixing or improve the miscibility between hydrophilic and lipophilic phases, forming a bi-continuous lipid bilayer. A Suitable amount of sodium stearate was introduced in cubosomal dispersion as a stabilizing agent by reducing the interfacial tension between the lipid and aqueous phases as well as imparting a negative charge. The two solutions were blended, and the resultant dispersion underwent homogenization using a High-pressure homogenizer (Panda Plus 2000, Niro savi, Italy) for 10 cycles at a pressure of 550-650 bar, followed by

sonication for 2 min. The preparation was permitted to reach equilibrium over 24 h before undergoing additional examination.

Table 4. Formulation Compositions of Haloperidol cubosomes

Formulation	Factor 1	Factor 2	Factor 3
	A: GMO (mg)	B: P407 (mg)	C: PVA (mg)
H1	0	-1	1
H2	0	-1	-1
H3	0	1	-1
H4	-1	-1	0
H5	1	0	-1
H6	1	0	1
H7	-1	1	0
H8	1	-1	0
H9	-1	0	1
H10	0	1	1
H11	1	1	0
H12	-1	0	-1
H13	0	0	0

Table 5. Actual values of coded levels

Independent variables	Coded Levels		
	-1	0	+1
GMO (mg)	500	600	700
P407 (mg)	50	60	70
PVA (mg)	10	20	30

4.5. Preparation of HPD Cubosomal in-situ Gel

First Step: A precisely measured quantity of 0.5% sodium alginate and 0.5% Artocarpus heterophyllus L. mucilage/Carbopol P934 was meticulously dispersed separately in distilled water.
Second Step: The resulting cubosomal colloid underwent stirring for 20 min. at 70-80 ^{o}C, employing a magnetic stirrer, then the mixture was cooled to reach room temperature (RT). Following this, a gradual addition of 2% Kolliphor 188 took place, accompanied by continuous stirring.

The initial and subsequent steps were combined by stirring for an additional 10 min. The resulting formulation was transferred into an amber-colored glass vial, sealed with a rubber closure, and stored securely in the refrigerator (4-8^{o}C) until further use. [22].

4.6. Characterization of HPD Cubosomal Dispersion

4.6.1. Assessment of PS, PDI and ZP

The particle size (PS), polydispersity index (PDI), and zeta potential (ZP) of prepared HPD cubosomes were determined using a Particle Size Analyzer (Zetasizer Nano ZS, Malvern Panalytical Ltd., Malvern, UK). The analysis has taken place at a temperature of 25℃ with a detection angle set at 90°. To ensure robustness and reliability, all assessments were conducted in triplicates (n=3), and subsequently, the average readings ± standard deviation (SD) were meticulously analyzed. This approach ensured a comprehensive and precise assessment of the key characteristics, facilitating a detailed understanding of the HPD cubosomal formulation. [7].

4.6.2. Assessment of Percentage (%) Entrapment Efficiency

The process of calculating the entrapment efficiency (EE) percentage for HPD cubosomes was executed with great precision. Sample (3 ml) from the formulation was centrifuged using an ultra-centrifuge (Optima Max-XP, Beckman Coulter, USA) at a rotational speed of 45000 rpm for 45 min. at 4°C. Post centrifugation, the supernatant samples were gently gathered and suitably diluted with PBS of pH 6.4. After the dilution process, the absorbance was recorded at the λ_{max} of 248 nm. The EE percentage was then computed using the given formula, which allowed for an accurate evaluation of the encapsulation efficiency of HPD in the cubosomes. The goal of this method was to accurately determine the formulation's ability to hold the therapeutic compound [23].

$$\text{Entrapment Efficiency (\%)} = \frac{(\text{Total Drug conc.} - \text{Supernatant Drug conc.})}{\text{Total Drug conc.}} \text{x}100 \quad (1)$$

4.6.3. Drug Content (%)

To estimate the total drug content in the formulation, Sample dispersion (1 ml) was dissolved in 10 ml of pH 6.4 PBS. Utilizing a UV spectrophotometer (1800, Shimadzu, Japan), the amount of HPD in each sample was determined by measuring the absorbance at the maximum wavelength (λ_{max}) of 248 nm. The application of the following equation facilitated the determination of the total drug content. This method ensured a precise and reliable assessment of the overall quantity of HPD present in the formulated dispersion. [23].

$$\text{Total Drug content (\%)} = \frac{(\text{Amount of drug in the Sample})}{(\text{Amount of Initial Drug Used})} \times 100 \quad (2)$$

4.6.4. TEM imaging of optimized HPD Cubosomal Dispersion

The HPD-loaded cubosomes were examined for their structural and morphological attributes using Transmission Electron Microscopy (Tecnai 12, FEI, USA). After diluting the sample (200 times) with double-distilled water, they were meticulously arranged on copper grids supported by Formvar films. The excess sample was gently wiped off with filter paper. This was followed by a brief staining period of 30 seconds in a solution of 0.5% phosphotungstic acid, after which the samples were left to dry before observation. The TEM, operating at 80 kV, provided high-resolution images, enabling a comprehensive analysis of the characteristics of the cubosomes [17].

4.6.5. In-Vitro Permeation Study

To conduct an in vitro drug permeation study of formulations (F1 to F13), we utilized a dialysis membrane that had been pre-soaked in pH 6.4 PBS for 24 h. The diffusion cell (Orchid, India) characterized a dialysis membrane positioned between the donor and receptor compartments (25 ml), with a magnetic stirrer maintaining a speed of 100 rpm in a water bath set at 37 ± 0.5°C. The receptor compartment was filled with pH 6.4 PBS. Introducing a precisely weighed amount of cubosomal dispersion into the donor chamber over the membrane, the process was carefully executed to eliminate air bubbles. Periodic withdrawals at intervals of 0.5, 1, 2, 3, 4, 5, and 6 h, accompanied by replacement with an equal volume of PBS, ensured the maintenance of sink conditions. Following suitable dilutions, samples were analyzed by UV-visible spectroscopy at the λmax of 248 nm to determine the drug diffusion profile [23, 24].

4.7. Characterization of Nasal Cubosomal in-situ Gel of HPD

4.7.1. pH

The digital pH meter (Rolex, India) was calibrated before assessing the pH of the in-situ gel, ensuring precision and accuracy in the measurement process. The targeted pH range, ideally between 4.5 and 6.5, aligns with the optimal nasal pH. In individual beakers, each formulation, measuring 20 ml, was cautiously deposited, and a glass electrode was submerged into the formulation samples. Triplicate measurements were conducted to ensure accuracy in determining the pH of the solution. This meticulous process adhered to the specified pH parameters, crucial for assessing the in-situ gel's compatibility with the nasal environment which in turn is crucial to understanding its performance [25].

4.7.2. Gelation Assessment

Aliquot (5 ml) of the HPD in-situ gel was carefully transferred into a beaker containing a magnetic bar and positioned on a magnetic stirrer. A nasal fluid substitute, mimicking the components found in nasal secretions (comprising 8.77 mg/ml NaCl, 2.98 mg/ml KCl, and 0.59 mg/ml $CaCl^2$ per 1000 ml), was slowly added while stirring continuously [22,23,32]. The gelation point was determined by observing the cessation of the magnetic bar's movement. The degree of cubogel gelation was graded as follows: (-) indicating no gelation, (+) denoting weak gelation that dissolves quickly, (++) representing immediate gelation lasting a few hours, and (+++) indicating immediate gelation persisting for an extended period. Additionally, (++++) represented a very firm gel. Employing this method facilitated a qualitative evaluation of gelation characteristics, offering valuable insights into the formulation's behavior.

4.7.3. Assessment of Viscosity

The viscosity analysis of the formulated gel, incorporating HPD, Artocarpus heterophyllus L. (AH) mucilage, and Carbopol P934 was conducted utilizing the Brookfield DV-PRO-II viscometer equipped with Spindle number-63, operating at 100 rpm (shear rate). Submerging the temperature-sensing probe into the gel, we meticulously recorded the temperature of the substance. Subsequently, assessed the viscosity at a temperature of 32 to 34°C, as outlined in reference [26]. This method allowed for a comprehensive comparison of the viscosity profiles, providing insights into the gel's rheological properties at physiologically relevant temperatures.

4.7.4. Determination of Gel Strength

The strength of the HPD nasal gel was determined using a texture analyzer (CT310K, Brookfield, USA). The assessment involves measuring the time (sec.) taken for a piston to penetrate across the gel at a distance of 5 cm [27]. This methodology provided a precise measurement of the gel's resistance to deformation, offering valuable information about its mechanical properties and structural integrity.

4.7.5. Determination of Mucoadhesive Strength

Assessing mucoadhesive strength involved measuring the force needed to separate the gel from the tissue of nasal mucosa, following established methodologies for precise and reliable evaluations [28]. Nasal mucosal tissues sourced from a local slaughterhouse were promptly employed for the assessment. The procedure consisted of affixing a nasal tissue section to the top probe using a fast-drying adhesive (cyanoacrylate). With an uncovered mucosal membrane area of 2.5 cm^2, a specific amount of each formulation sample was applied to the bottom probe. The probe carrying the nasal tissue was gradually brought down till contact with the sample surface was established. Applying a force (0.1 N) ensured optimal contact time between the nasal mucosa and the optimized sample. The mucoadhesive strength, determined as separation force in dyne/cm^2, was calculated based on the minimum weights required to separate tissues from the sample surfaces and provided an equation used to calculate force for detachment. This method not only quantified the gel's ability to adhere to and interact with nasal mucosal tissue but also provided a robust measure of its mucoadhesive strength.

Mucoadhesive strength (dyne/cm^2) = w. g/SA

Where;
w: weight requires for separation of gel from nasal mucosal tissue, g: the acceleration due to gravity (980 cm/s^2), and SA: the surface area of the nasal mucosa of sheep.

4.7.6. Ex-Vivo Study

Fresh nasal mucosa collected from the olfactory region of sheep was submerged in PBS with a pH of 6.4. Placed on diffusion cells at 34°C, PBS pH 6.4 was transferred to the receptor chamber of the Franz diffusion cell apparatus. The donor chamber inclusive of formulation equivalent to 1 mg of HPD with AH mucilage and carbopol P934, and comparisons were made at specific time points over 6 h. Samples (1 ml) were withdrawn from the receptor compartment, with each withdrawal replaced by PBS pH 6.4. The analyzed samples, obtained after filtration, underwent assessment using a UV-visible spectrophotometer set at 248 nm to quantify the extent of drug permeation [8, 29].

4.7.7. Nasal Histopathological Study

Fresh nasal mucosa from sheep, preserved in 10% formalin, underwent an ex vivo drug diffusion investigation utilizing HPD cubosomal gel in a pH 6.4 PBS solution. The selection of a buffer system was made as per the pH of the nasal cavity (range as 4-6 pH) to mitigate potential irritation. For the negative control, pH 6.4 PBS was chosen, while isopropyl alcohol served as the positive control to assess any adverse effects on sheep nasal mucosa. Histological examinations were carried out on both untreated and treated mucosa. These samples were stained with eosin and observed under light microscopy (Motic DMW B1-223ASC) to detect any harm or injury to the tissue resulting from the exposure to 1 ml of the optimized gel over 6 h [31].

4.7.8. Stability study

The stability investigation aimed to comprehensively evaluate how the formulation additives influence both drug stability and the physical integrity of the optimized formulation during storage under specific humidity and conditions. The selected HPD cubosomal dispersion underwent rigorous stability

testing, being stored at a controlled temperature of 4 ± 2 ℃ within a stability chamber. At defined intervals of 0, 30, 60, and 90 days, samples were withdrawn to analyze the impact on crucial characteristics like particle dimensions, Polydispersity Index (PDI), and zeta potential were evaluated. [15]. This systematic approach allowed for a thorough examination of the formulation's resilience and performance over an extended duration of storage, providing valuable insights into its stability profile.

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