RESEARCH PAPER

A nanoparticle loaded nasal sol-gel system of midazolam hydrochloride for the management of intermittent seizures

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ABSTRACT

Objective(s): Midazolam hydrochloride is a short-acting hypnotic sedative with anticonvulsant properties. Considering the poor bioavailability, rapid elimination, and inconvenience of administering oral medication in epileptic conditions, this study proposed the use of a nanoparticulate in situ nasal gel system for midazolam to achieve an optimum therapeutic effect.

***Materials and Methods*:** Drug-loaded PLGA nanoparticles were prepared by an emulsion-solvent evaporation method utilizing a Box–Behnken design concentrated on particle size, zeta potential, and drug entrapment efficiency as responses. The optimized nanoparticles were incorporated into a 0.2% w/v gellan gum solution. The nanoparticle-loaded gel was studied for *ex vivo* drug permeation through excised sheep nasal mucosa. The anticonvulsive activity of the gel was compared with that of the marketed midazolam nasal spray (0.5 mg/0.1 ml).

Results: Particle size (200 nm to 310 nm), zeta potential (-25mV to -31mV), and entrapment efficiency (81.23±0.21 to 93.32±0.28%) were observed in the formulations. The drug release from the formulations over 12 h was found to be 75.32±0.028to 84.63±0.061%. Particle size and zeta potential were best fitted to the quadratic model, and an entrapment efficiency linear model was suggested. The *ex vivo* permeation studies of the optimized nanoparticle-incorporated gel formulation exhibited a fourfold increase in flux and permeability coefficient in comparison to the pure drug-incorporated gel. The anticonvulsive effect in pentylenetetrazol (PTZ)-induced rat model epilepsy showed a similar anticonvulsive profile to that of the marketed nasal spray.

Conclusion: Drug-loaded nanoparticles incorporated in situ gel would be a promising, biocompatible, and non-invasive approach to achieve the required therapeutic efficacy by sustained and direct action on brain cells

Keywords: *Nasal, Gellan gum; Midazolam; Nanoparticles; PLGA*

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INTRODUCTION

Epilepsy is the fourth most common neurological problem reported by the World Health Organization and affects individuals of all age groups. When two or more unprovoked seizures occur, the patient is diagnosed with epilepsy [1]. There are chances of intermittent seizures in epileptic conditions, which are characterized by continuous or repetitive seizures in the brain, each lasting five minutes or more for up to two hours, without regaining consciousness between seizures [2, 3].

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At present, the available treatments for seizures focus on prevention and control rather than cure. Despite existing treatment options, the risk of seizure occurrence and subsequent complications remains, potentially affecting quality of life. Although antiepileptic drugs (AEDs) can often manage epileptic seizures, they do not offer a definitive cure, as AED dosages typically require individual adjustment. A significant number of patients with epilepsy fail to respond to first-line medications. Consequently, pharmacological interventions aim to manage symptoms through long-term administration of antiepileptic drugs [4].

Individuals with epilepsy are administered medications in urgent situations or for short- and long-term treatment. Urgent cases necessitate prompt intervention; in other instances, there is a

need to minimize adverse effects to enhance patient adherence. Treating epilepsy with traditional methods presents challenges owing to limited bioavailability and the development of drug resistance, which diminishes the effectiveness of ongoing treatments [4].

Several methods have been investigated for the delivery of epilepsy medication. While oral administration is common, we sometimes encounter nil-by-mouth conditions, where patients cannot take or swallow medications by mouth. Intravenous (IV) administration is a well-studied alternative but requires trained healthcare professionals for proper implementation. In cases where access is restricted, alternative routes of administration, such as rectal and buccal, have been utilized. However, these methods have limitations in their application [5].

The challenges in epilepsy management stemming from the inability of current antiepileptic drugs (AEDs) to effectively cross the blood-brain barrier (BBB) can be addressed through appropriate drug delivery mechanisms. An ideal system would enable targeted and regulated AED administration to specific brain regions, thereby minimizing drug-related side effects and improving therapeutic efficacy [5].

Research on intranasal administration of antiepileptic drugs (AEDs) is gaining momentum, with several benzodiazepine nasal sprays currently in clinical trials and a few already approved for use. This delivery method may utilize multiple pathways, including the trigeminal and olfactory nerves, as well as a systemic route. Each of these pathways could contribute to drug efficacy, with the systemic route potentially facilitating passage through the blood-brain barrier (BBB). This administration method typically yields a lower effective dose than alternative routes, and early results from intranasal delivery have been positive and show potential [6].

Nasal administration offers several benefits over oral delivery, including a quicker action, reduced drug breakdown, and enhanced absorption. Compared with intravenous methods, nasal administration allows for easier self-administration, greater patient acceptance, and direct drug transport to the brain by circumventing the blood-brain barrier through olfactory nerve pathways.

Among the drug delivery devices, nasal sprays are the most common. They offer benefits, such as ease of use and cost-effective production. Nevertheless, they have drawbacks including imprecise dosing and inadequate depth of drug administration. Additionally, the limited time the medication remains in the nasal cavity due to quick mucociliary elimination negatively affects its bioavailability [7].

In situ nasal gels have emerged as promising alternatives to conventional gels and mucoadhesive powders, addressing their limitations and offering potential for both localized and systemic drug administration. These innovative drug delivery systems initially exist in a liquid state prior to application. However, upon administration, they transform into a gel-like consistency in response to specific microenvironmental conditions such as temperature fluctuations, pH alterations, or the introduction of ions [8].

Recently, various nanostructured drug delivery systems have been shown to effectively control epilepsy by addressing the challenge of antiepileptic drug (AED) elimination at the blood-brain barrier (BBB) and improving drug bioavailability. Employing customized nanocarriers to direct drug molecules to the brain is a novel approach to selective brain drug targeting. Therefore, an optimal strategy for enhancing drug retention and targeting is to integrate nanotechnology with in situ gel technology [[9,10].

Benzodiazepines, play a pivotal role in managing epilepsy by potentiating neural inhibition mediated by gamma-aminobutyric acid (GABA) receptors [11]. Midazolam hydrochloride, a derivative of benzodiazepine, was approved by the United States Food and United States Food and USFDA in 2018 for epilepsy treatment. It is marketed as a nasal spray for the management of acute, recurring, and stereotypical seizure episodes. The drug exhibits 27 % bioavailability and a 2.5-hour elimination half-life when taken orally [12-14].

Although midazolam is currently available as a nasal spray, which is expected to surpass conventional nasal formulations, this study proposes an in situ nasal drop formulation. This new approach aimed to extend the retention time of midazolam in the nasal cavity and incorporate nanotechnology for additional benefits. These advantages include controlled drug release and improved drug transport across the blood-brain barrier (BBB) due to the size range of the particles. Therefore, this study proposes a convergence intranasal route, an in situ approach using natural polymer and nanoparticle technology with

biodegradable polymeric space to manage epileptic conditions.

MATERIALS AND METHODS

Materials

The Mylan laboratories extended their assistance in providing midazolam. PLGA polymer, poloxamer 407, and gellan gum were procured from Sigma Aldrich Corporation. Analytical-grade solvents and other chemicals were of analytical grade.

Compatibility studies-Fourier-Transform Infrared Spectroscopy (FTIR)

Midazolam and the physical mixtures were kept on a sample holder, IR spectra (using Bruker Alpha II FTIR instrument recorded using Attenuated Total Reflection (ATR) mechanism was employed to record the IR spectra at 400-4000 cm-1 at a resolution of 1 cm-1 [15,16].

Preparation of drug loaded nanoparticles

Emulsification-solvent evaporation method: The polymer solution (in acetone) was added dropwise by syringing to 10 ml of an aqueous drug-surfactant solution (Poloxamer 407 1% (w/v) solution on a high-pressure homogenizer at 10,000 rpm for 10 min in an ice bath. The homogenized nanosuspension obtained via ultrasonication was freeze-dried to obtain nanoparticles. [15,16].

Optimization of formulation trials using Design-Expert® Software Version 13

A Box-Behnken design (BBD) used to statistically optimize the formulations. The factors (X1-polymer concentration, X2; Surfactant concentration X3; phase volume ratio) for the responses are listed in (Table 1). Fifteen runs in a random order (Table 2) consisted of three replicates and a central point [17,18].

Entrapment efficiency

Nanoparticles (25mg) was added to 10 ml water in Eppendorf tubes, subjected to vertexing followed by centrifugation at 5000-15000rpm for 50 min, after diluting the supernatant with distilled water, the absorbance was measured at 219 against water as blank. [19].

$$Entrapment\ efficiency\ = \frac{Total\ drug\ content - free\ drug\ content * 100}{Total\ drug\ content}$$

Table 1. Factors and responses selected in Box Behnken design

Factors	Ranges	
	Low level	High level
(X1) Polymer conc. (mg)	100	500
(X2) Surfactant conc. (mg)	150	300
(X3) Phase volume ratio (%)	20	40
Responses	**Lower limit**	**Upper limit**
(R1) Drug entrapment efficiency (%)	80	90
(R2) Particle size(nm)	50	600
(R3) Zeta potential (mV)	≥-30	

Table 2. Formulations suggested by the experimental design

Formulation code	Drug (mg)	Polymer concentration (mg)	Surfactant concentration (mg)	Phase volume ratio (%)
T1	250	500	150	30
T2	250	100	300	30
T3	250	300	150	20
T4	250	300	225	30
T5	250	300	300	20
T6	250	500	225	20
T7	250	300	225	30
T8	250	500	225	40
T9	250	300	225	30
T10	250	100	225	20
T11	250	100	225	40
T12	250	300	300	40
T13	250	100	150	30
T14	250	300	150	40
T15	250	500	300	30

Particle size analysis and zeta potential

The mean particle size distribution and zeta potential were determined via SZ-100 HORIBA via dynamic light scattering (DLS) and electrophoretic methods in a zeta measurement cell, respectively. The diluted samples were analyzed at a scattering angle of 90° and 25.2 °C. The electrophoretic mobility of the nanodispersion was measured by laser Doppler velocimetry at 25°C [20].

Drug release profile

In vitro experiments were conducted using a diffusion cell setup. Approximately 25 mg of nanoparticles was held on a dialysis membrane between the two compartments of the diffusion cell. The donor side had (nanoparticles and 1 ml of (SNF), while the receptor side (had 50 ml of simulated nasal fluid at pH 6.4). The receptor compartment had a constant temperature of 35°C, and was magnetically stirred at 120 rpm. For a 1 ml volume of receiver fluid drawn on an hourly basis, an equal volume of SNF was added to the receiver compartment to ensure sink conditions. The drug concentration was determined spectrophotometrically after appropriate dilution at a wavelength of 219 nm [21].

Statistical treatment of the data

All three responses, such as particle size, zeta potential, and drug entrapment, were statistically analyzed using Pearson's correlation coefficient.

Optimization of formulation trials

After evaluating the three responses using statistical principles and understanding the design space through graphical methods using the desirability criterion, the optimum formulation was selected

Scanning Electron microscopy (SEM)

After sputtering the dispersion with gold, the samples were spread onto a sample holder. Images were obtained at a voltage of 30 KV under argon purging [22].

Differential scanning calorimetry (DSC)

Approximately 5 mg of midazolam, the physical mixture, and the formulation were precisely placed in non-hermetically sealed aluminium pans and crimped. Constant heat was supplied (0°C to 350°C) at 10 °C/min with nitrogen purging at 40 ml/min; the DSC thermograms were recorded using Shimadzu DSC-60, Japan [20].

Preparation of nanoparticulate in situ nasal gel

The study utilized deacetylated gellan gum at concentrations of 0.05, 0.1, 0.2, 0.3, and 0.5% w/v. The gelling properties and viscosities of these concentrations were recorded using SNF at 34°C [23]. Midazolam-loaded nanoparticles (equivalent to the prescribed dose of 5 mg/mL, weighed according to its drug content) were mechanically mixed with gellan gum solution (0.2%w/w) to form a nanoparticle-loaded in situ gel [24].

Evaluation

pH and Viscosity

pH measurements were performed using a glass electrode. Viscosity imparts retentive properties to nasal mucosa. Viscosity measurements were carried out in triplicate at angular velocities of 50–100 rpm at 37 °C using a brook-field viscometer [24].

Drug content

A one ml volume of gel was placed in a 10 ml volumetric flask, suitably diluted with double distilled water, and spectrophotometric analysis of the sample was carried out at 214 nm [24].

Ex vivo permeation studies

Fresh nasal mucosa from sheep, with its olfactory region removed, was placed into the SNF. The samples were then positioned between the two compartments of the assembly, referred to as the donor and receiver, which contained phosphate buffer solution at pH 7.4. After adding the nanoparticulate gel to the donor side, samples were withdrawn at suitable time intervals, analyzed spectroscopically, and the drug concentration was calculated [24].

Stability study

Two stability conditions were used in this study: 40 ^{o} C ± 2 ^{o} C 75 ± 5% relative humidity, and (25 °C ± 2) 65 ± 5% relative humidity. The gel was studied for its viscosity, drug content, and pH.

Anticonvulsant activity

All animal experiments comply with the committee for the purpose of control and supervision of experiments on animals (CPCSEA) guidelines and were carried out in accordance with prevention of cruelty to animal (PCA) Act, 1960. Healthy Wistar rats (150-200 g) (maintained by CPCSEA guidelines) of either sex, provided by animal house of the institution. The animals were divided into IV groups (n=3 rats per group). Group I received (saline) and group II received standard drug (PTZ). Group III, Nanoparticle in-situ gel (5 mg/1 ml), and Group IV, marketed formulation of midazolam nasal spray (Medistat, Alteus Biogenics) (0.5 mg midazolam/0.1 ml each metered dose in single spray). The volume of the 0.005 ml (0.5 mg

Table 3.Percentage yield and physical observation of blank formulations

Trial no.	PLGA (mg)	Poloxamer 407 (%)	Percentage Yield (%)	Observation
1	100	0.1	80.12	Slightly sticky
2	100	1	65.14	Slightly sticky
3	100	0.3	82.56	Free flowing
4	500	0.2	61.66	Free flowing
5	500	0.5	34.12	Free flowing
6	500	1	48.2	Free flowing
7	700	0.7	40.65	Free flowing
8	500	0.5	43.06	Free flowing
9	700	0.9	36.95	Free flowing
10	500	1	33.99	Free flowing
11	500	0.3	88.15	Free flowing

midazolam /0.1 ml) of marketed nasal spray and 0.003 ml of nanoparticulate in situ drops were instilled into each nostril animal using a micropipette. Convulsions were induced by administering pentylenetetrazole (PTZ) via the intraperitoneal route at a dose level (40 mg/kg) immediately after administration of the formulations. Parameters such as the onset of seizures, duration, and recovery time were observed, and the data were statistically analyzed using ANOVA and the Brown-Forsythe test [25].

RESULTS AND DISCUSSION

Preparation of Nanoparticles

Initially, blank emulsions were prepared to determine the effect of the concentration of the polymer (PLGA), surfactant (poloxamer), and phase volume ratio (organic to aqueous phase) on the formation of nanoparticles and their physical properties, such as flowability and % yield, as shown in Table 3. In preliminary trials, the product obtained was sticky and non-free-flowing. The glass transition temperature of the polymer has been reported to be 45°C. The introduction of heat into the emulsion system at a high speed might have converted the polymer to a rubbery stage, and thus, changes were observed physically. Drug-loaded nanoparticles were prepared under cooling conditions and evaluated for drug content, entrapment efficiency, and particle size.

Evaluation of the responses and statistical treatment of the data

Particle size and distribution determined by DLS ranged from 212 nm to 309 nm, and the poly dispersibility index of all formulations was less than 0.162. The particle size and distribution patterns indicated that the influence of the formulation variables and processing conditions was suitable for obtaining nanosized particles. A low polydispersity value indicates minimum aggregation of nanoparticles and their consistency during manufacturing. Drug concentration had a considerable impact on particle size. A higher concentration (500 mg) of PLGA increased the particle size to 309 nm, as in formulation T15.

Similarly, the observed particle size was lower than 212 nm when using a lower polymer concentration. The larger particle size at higher concentrations may be attributed to viscous forces, which result in the coagulation of droplets on emulsification and, thus, on drying, resulting in larger particles. This variation in the size range may also be due to the effect of polylactic and glycolic acid concentrations on the particle size. The phase volume ratio had an impact on the particle size. The particle size was larger when the phase volume ratio was 40 %, as in T11, T12, and T14. However, the phase volume ratio and polymer concentration could affect particle size, as observed in the other formulations.

The zeta potential indicates the net surface charge and its primary factor that determines the stability of the colloids. The zeta ranged between -25 mV and -31 mV, which means that the nanoparticles offer relatively good stability under the chosen processing conditions. The negative zeta potential was attributed to the terminal carboxylic acid group. A five-mV difference was observed from the T3 formulation, with a maximum particle size of 212 nm, to the T15 formulation, with a particle size of 309 nm. This effect may be due to the larger particle size; therefore, a low charge density on the surface resulted in fewer repulsive forces.

Drug entrapment efficiency ranged from 81.23±0.21 to 93.32±0.28%. The entrapment efficiency was greatly affected by the polymer, surfactant concentration, and phase volume ratio; in T2, T10, T11, and T13, the polymer concentration slightly affected the entrapment efficiency, whereas no notable changes were observed by changing the surfactant amount. The phase volume ratio considerably affected entrapment efficiency, as formulations T8, T11, T12, and T14 had relatively lower entrapment efficiencies. However, drug entrapment efficiency was not affected by phase

Table 4. Results of experimental trials

Formulation code	Particle size (nm)	Zeta potential (mV)	Drug entrapment efficiency (%)
T1	251	29	90.32±0.23
T2	233	28	85.04±0.112
T3	212	31	90.33±0.23
T4	213	28	88.62±0.20
T5	275	28	91.52±0.19
T6	271	30	93.03±0.25
T7	220	29	89.12±0.18
T8	292	28	93.32±0.28
T9	213	27	85.2±0.25
T10	215	30	86.03±0.18
T11	216	29	83.23±0.23
T12	267	28	88.06±0.23
T13	212	28	85.23±0.26
T14	267	28	81.23±0.21

volume alone. Instead, it was also affected by the cofactor; the concentration of the polymer, as in the T8 formulation, had a high drug loading, which used the highest polymer concentration. When the phase volume ratio was 20%, as in T3, T5, T6 and T10, the drug entrapment efficiency was 90.33±0.23, 91.52±0.19,.03±0.25 and 86.03±0.18 % respectively. However, at T10, the lowest concentration of the polymer might have reduced the entrapment efficiency to 86.03±0.18%. Higher phase volume ratios lead to a large concentration of the organic phase; thus, emulsification results in higher resistive forces, resulting in larger particles and lower entrapment efficiency. (Table 4)

All research findings obtained were subjected to statistical analysis via correlation to understand the influence of each parameter on the other parameters. The correlation typically has values ranging from -one to zero–one. The results indicate that the Pearson correlation coefficient of -0.4326 between particle size and zeta potential shows that particle size has an inverse effect on zeta potential, and vice versa. The correlation between particle size and entrapment efficiency was 0.460432, indicating a linear relationship between both responses.

In vitro drug release studies

The *in vitro* drug release from the formulations at 12 h ranged from 75.32±0.028 to 84.63±0.061% shown in Fig.1; for the first half an hour, approximately 8.27±0.03 to10.76±0.06 % drug release was observed, whereas over seven h, 50.6±0.032 % to 59.9±0.012 % drug release was observed. In the next 7 h, approximately 30 % of the drug was released from various formulations on an average. A rapid initial drug release in 30 min accounted for the surface drug, and a sustained effect was observed in the later phase. The slow and sustained release of drugs from NPs was due to slow degradation/erosion from the backbones after fluid entry and the slow degradation and erosion of the backbone. An ANOVA was carried out for the *in vitro* drug release data, and the results indicated that there was no significant difference between the results, as supported by P (0.504≥0.05).

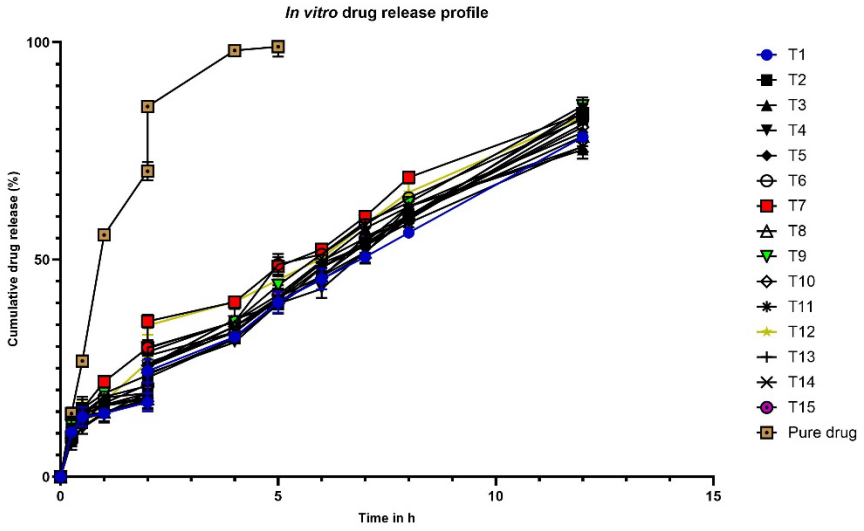

Fig. 1. *In vitro* drug release profile of drug loaded formulations (T1-T15)

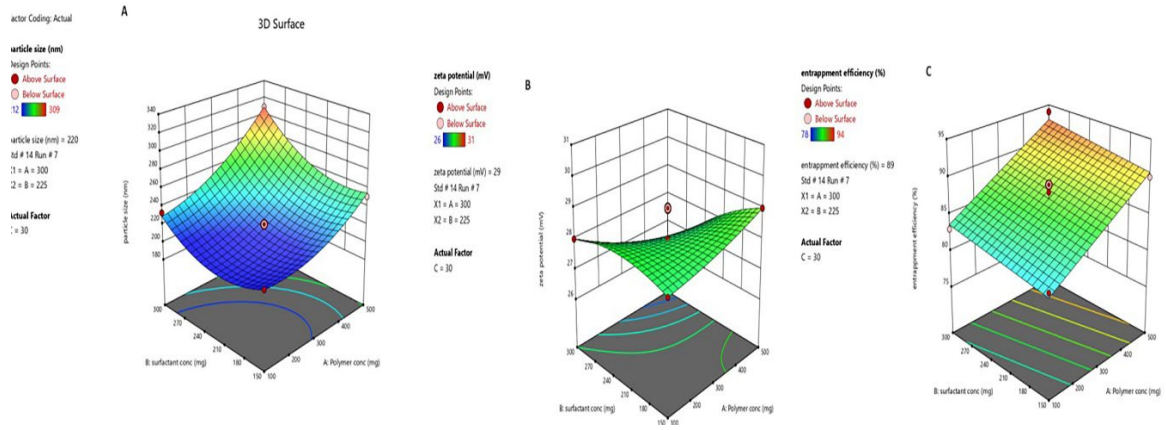

Fig. 2. 3D response surface of (A) particle size (B) zeta potential (C) transmittance

Responses evaluation

To understand and identify combinations of variables or factors that jointly optimize a response, a response evaluation was conducted. This will be used to analyze the impact of multiple factors on a selected response. Each response will be analyzed separately using a fit model concept. A "Box Behnken design" was used in optimization trials (factors – polymer concentration, concentration, concentration of surfactant and phase volume ratio) for outcomes such as size, zeta potential and drug entrapment efficiency—the experimental design generated 15 experimental trials. The model fit summary suggested a quadratic fit for particle size and zeta potential and a linear model for entrapment efficiency (P<0.05).

The polymer concentration significantly affected the particle size (P<0.0001), and a positive effect was observed. Similarly, surfactant concentration significantly affected the particle size and had an inverse effect on the particle size. (P<0.004). However, the interactive effect of these two factors had a positive impact on the particle size distribution. As the phase volume ratio increased, particle size decreased. A similar observation was made for the surfactant concentration. The polymer concentration and phase volume did not significantly contribute to the particle size distribution, although they had a positive effect. All except the surfactant and phase volume interactions brought large particles into the emulsification. The quadratic effect of all three factors was significant for the particle size P<0.05).

The surfactant concentration and phase volume ratio significantly affected the zeta potential. (p <0.0202). High-order interactions were observed only in the phase volume ratio of the zeta potential. The remaining factors had no significant effects. All parameters except the phase volume had a positive impact on the zeta potential. The interactive effect between the polymer and surfactant and the polymer and phase volume increased the zeta potential, but it was not significant.

The polymer concentration and phase volume ratio significantly affected the drug-loading efficiency; all the factors had a linear relationship, and a high-order effect was observed. The other two factors increased the drug entrapment efficiency, except for the phase volume ratio. (Fig.2.)

A desirability approach of 0.91 aided in selecting the formula with a polymer concentration, surfactant concentration and phase and volume ratio of 300 mg, 225 mg, and 30%, respectively.

Evaluation of optimum formula

The optimum formulation (at a concentration of 300 mg of polymer, 250 mg of surfactant, and phase volume ratio of 30) suggested a maximum desirability of 1, particle size of 220 nm, zeta potential of- 29mV and drug entrapment efficiency of 89 %. Optimum Formulation. The optimized formula had a particle size of 216.1 nm, zeta vale of 30.2Mv, 94.3±0.09 % drug content, and 89.23± 1.12 % entrapment efficiency, respectively. (Fig.3 and Fig. 4.) The results suggested that the optimum formulation was in the nanosized range, and that the predicted particle size was almost achieved. The formulation exhibited good stability, as the zeta value was ≥ 30 mV, suggesting minimal aggregation of the particles. Drug loading was found to be almost the same as the predicted value.

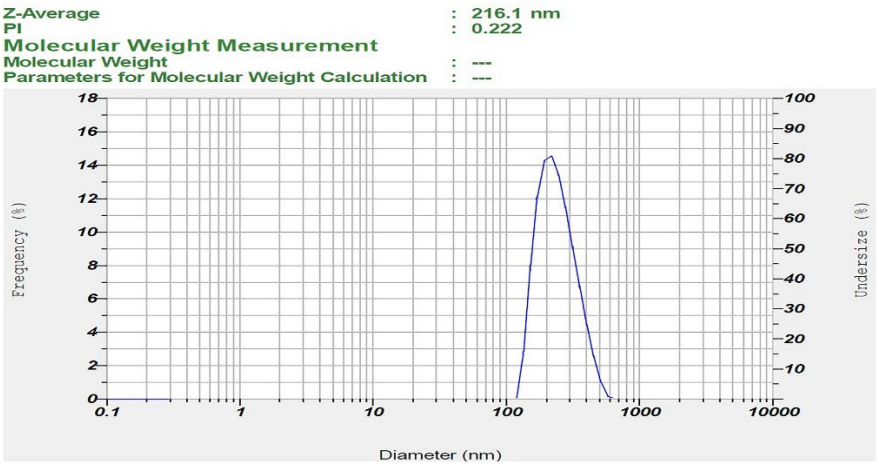

Fig. 3. Particle size of the optimum formulation

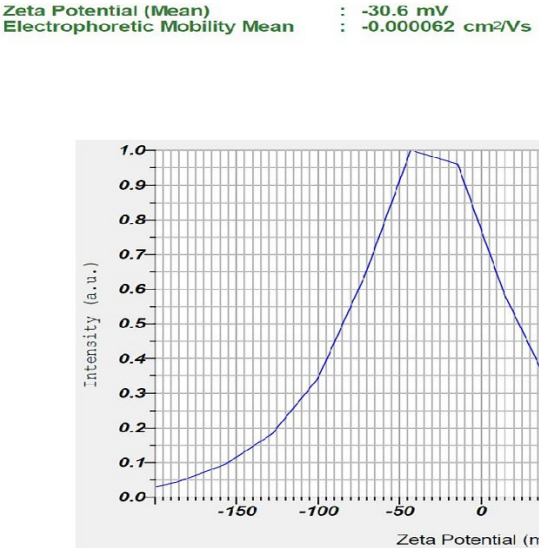

Fig. 4. Zeta potential of the optimum formulation

The percentage biases of 1.8, 4.1, and 0.25, for particle size, zeta potential, and drug entrapment efficiency, respectively, indicate that there was no significant difference between the predicted and observed results.

In nanotechnology, particles range between 1-100 nm are referred to as nanoparticles. A size range of 100 nm -300 nm is ideal for brain delivery via the BBB. In addition, for polymeric nanoparticles, the range recommended via the olfactory region 5 nm -200 nm. Therefore, the results of all the formulation trials and the optimum formulation's particle size suggest the suitability of these to cross both the olfactory region and BBB to the brain. The zeta values were related to particle size. There is an introduction surface charge density due to nanosization, and therefore, the chances of agglomeration are higher. Nevertheless, the zeta values were promising, and the results indicated good stability of the system.

Scanning Electron Microscopy

As observed Fig 5. The optimized drug-loaded nanoparticles were nearly spherical. Discrepancies and uniformity were evident among the particles. Zeta potential values of approximately, in turn, supported this -30mV, indicating the absence of mere aggregation of particles.

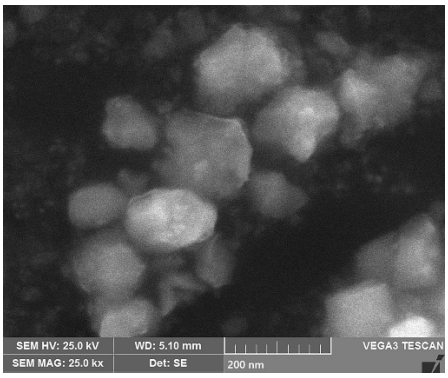

Fig.5. SEM photograph of nanoparticles

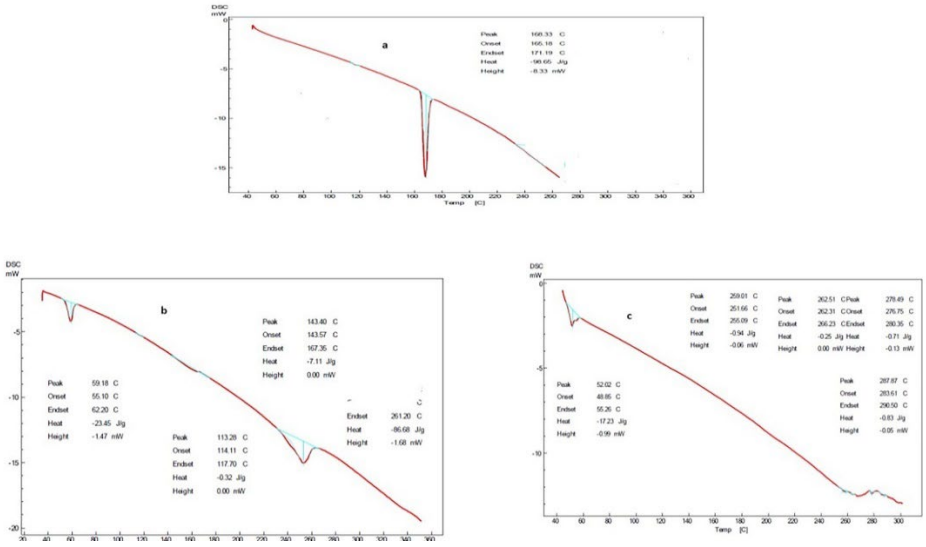

Fig. 6. DSC thermogram of (a) Pure drug (b) Physical mixture (C) Optimised nanoparticle incorporated gel

Differential scanning colorimetry

The DSC thermo-grams of Midazolam Hydrochloride, a physical mixture of drug PLGA, Poloxamer 407, gellan gum and nanoparticles were analyzed to study the existence of interactions, if any, between the drug and excipients in the nanoparticles. DSC thermogram of pure Midazolam Hydrochloride exhibited endothermic thermal transitions at 164°C (Fig. 6) corresponding to its melting temperature (Tm) (curve a), whereas in graph b, the same peak is observed at 143.4°C; perhaps the drug is more solubilized in the surfactant, and still, the endotherm 260–261°C, which corresponds to its melting temperature of PLGA (curve c). The thermogram of drug-loaded nanoparticles exhibited endotherms corresponding to the melting endotherms of the PLGA polymer (curve b), poloxamer at 59.180 °C, and gellan gum at 113.280 °C. The drug peak was absent in thermogram C, whereas the PLGA peak was 259.01°C and the poloxamer peak was 52.02°C. The two additional peaks in the thermogram may be due to the degradation products of gellan gum at very high temperatures.

Gelation studies with deacetylated gellan gum

The in situ gelling properties of (0.05%w/v-0.2%w/v) claim that gelling was very low at low concentrations of gellan gum; from 0.2%w/v concentration, gel formation was observed, and hereafter, the firmness of the gel increased with an increase in the concentration of gellan gum. (Table 5). The viscosity of the gel formulations increased to 0.4%w/v. However, the firmness of the gel was taken as a cut-off point, and after this concentration, the gel was very firm and the viscosity decreased. Based on these results, 0.2% w/v gel was selected to incorporate the optimized nanoparticle formula. As reported in the literature, the concentration of gellan gum affects gelation, and an optimum concentration is required to produce gel formation with both mono-and divalent ions present in SNF, which bridges the polymer chains.

Evaluation of Nano particle loaded in situ Gel formulation

A colorless gel formulation using 0.2%w/v gel was used to incorporate nanoparticles at a dose of 5 mg /0.1 ml. The nanoparticle gel had a drug content of 94.3±0.09%, pH of 5.9±1.02, and viscosity of 109±0.102 (cP).

Table 5. Gelation studies

Sl No	Concentration of Gellan	Viscosity (Cps)		Gelation in presence of
No	Gum (%W/V)	Without SNF	SNF	SNF
G1	0.05	21.4 ±0.103	41.4±0.12	No Gelation
G2	0.1	23.9±0.51	57.8±0.32	Slight Gelation
G3	0.2	30.6±0.03	114.0±0.21	Firm gel formation
G4	0.4	32.6±0.12	650.2±0.012	Thickened gel

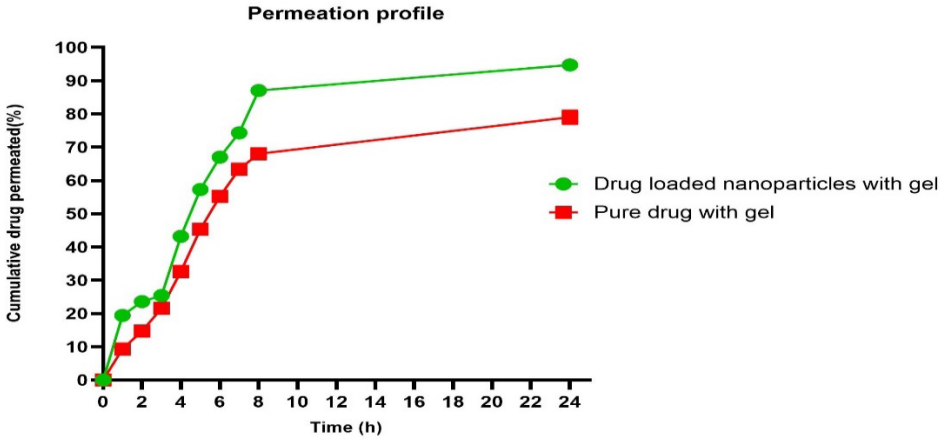

Fig. 7. *Ex-vivo* permeation studies

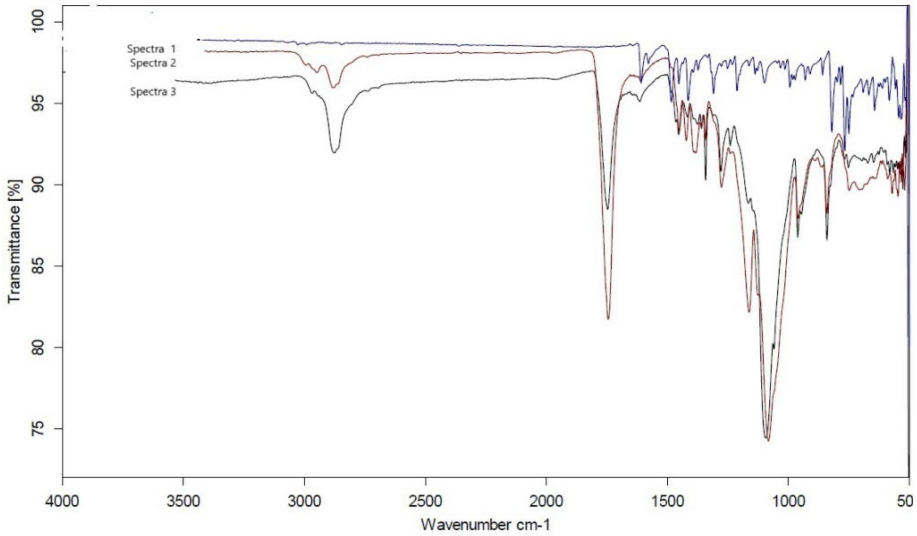

Fig. 8. FT-IR spectrum- Spectra 1) pure Midazolam Hydrochloride Spectra 2) physical mix Spectra 3) Nanoparticulate gel

Ex-vivo permeation profile

The rate of permeation was 72±0.20 and 68.02±0.05 in the first seven h, 87.02±0.23% and 94.72±0.20 % in 24 from drug-loaded gel and nanoparticle-loaded gels, respectively. There was a five-fold increase in the average flux and seven-fold increase in the permeability coefficient values of the nanoparticulate gel compared to the pure drug gel. The nanoparticles formulation showed activity up to 94.72±0.20 in 24 h against 79.02±0.02%, confirming prolonged gel activity. (Fig 7.)

FTIR spectrum

The IR spectra of Midazolam Hydrochloride revealed characteristic absorption bands in the following IR regions: drug peaks at 3073 cm-1 (N-H stretch), 2847(C-H stretch), 2947 cm−1 carbonyl stretch), 1642 cm-1 C-N stretch, and 533 cm-1. C-Cl stretch and 972 cm-1 C=C bending alkenes. Repetition of these bands in the physical blend and formulation spectra showed agreement with the drug and excipients (PLGA, poloxamer, gellan gum), as shown in Fig. 8.

Short term stability studies

The Midazolam Hydrochloride-loaded optimum formulation was subjected to short-term stability studies for 90 days under the aforementioned conditions. The viscosity and pH results showed that there was no change under different storage conditions. The maximum drug content was 93±0.02 % at 25 ±2°C RH and 91±0.13 at 40±2°C RH. This may be due to the retention of the amorphous matrix drug shelf life, which minimizes the expulsion of the drug during storage

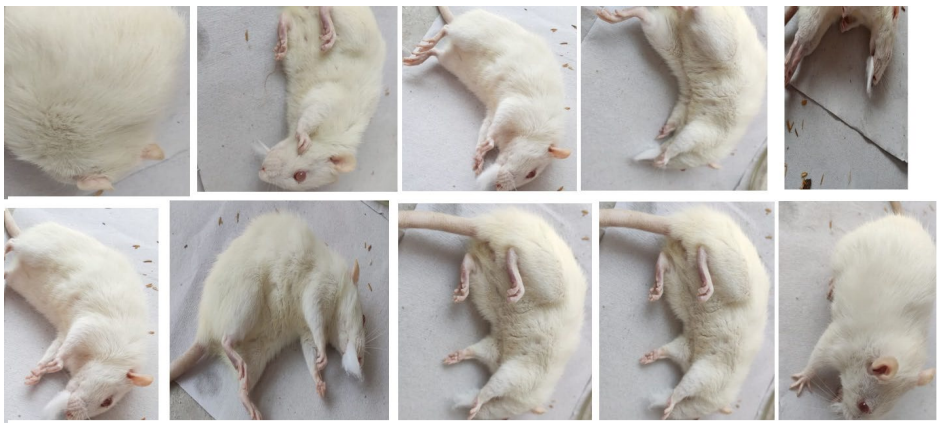

Fig. 9. Various stages of seizures observed after intraperitoneal administration of PTZ

Table 6. Results of anticonvulsant activity

Treatment Groups	Onset of seizure after administering PTZ (Sec)	Seizure duration (sec)	Seizure (%)	Supine Recovery (min)
Saline	-	-	-	-
PTZ	60± 2.12	306± 1.35	100	10
Nanoparticulate in situ gel	182±2.34	192±3.23	20	5
marketed formulation	180±1.16	186±2.25	20	5

Anti-convulsive activity

The nanoparticle-loaded in situ gel was studied for its anticonvulsant activity against the marketed midazolam nasal spray in rats with PTZ-induced seizures. (Fig. 9) The onset, duration, and recovery of seizures were observed. (Table 6) The seizure onset started from 60± 2.12 sec for the induced group, whereas 182±2.34 and 180±1.16 sec for the formulation-induced and marketed spray group, indicating the in-situ drops' potential to reduce seizure initiation. The seizure duration was 1.6 times less in the formulation group, which was on par with the marketed spray group. The % protection of the formulation in the control group was 62%, and the % protection was the same for the formulation compared to the reference group. The pharmacodynamic responses obtained were subjected to statistical analysis using one-way ANOVA. The P value (time of onset seizure < 0.001 and duration of seizure0.0001) indicates that the results are statistically significant, followed by Brown Forsythe's test to confirm ANOVA ($p< 0.05$).

CONCLUSION

In the present study, Midazolam Hydrochloride Nanoparticles were developed for intranasal application to provide the additional benefit of sustained drug action and direct action on brain cells. Drug-loaded PLGA nanoparticles were successfully prepared using the emulsion-solvent evaporation method. Characterization studies suggested that the methodology adopted and the processing conditions significantly affected the particle size, zeta potential, and entrapment efficiency. The surface response methodology ''Box-Behnken design model' successfully optimized the formulation trials. Drug-excipient interaction studies using IR and DSC proved that the excipients were safe and compatible. The comparative *ex-vivo* profile of the nanoparticle-incorporated gel vs. the pure drug gel confirmed its potential for permeation via the nasal mucosal membrane and further drug action. The anti-convulsant activity study in experimental rats proved the developed formulation's 'me too' action in comparison with the marketed nasal spray. Therefore, this study concluded that the nanoparticulate colloidal drug delivery system of Midazolam Hydrochloride using PLGA, poloxamer 407, and gellan gum is expected to provide an alternative to drug therapy via the nasal route to the brain, with minimal trouble to the patient from an administrative point of view.

ETHICAL CONSIDERATIONS

The approval for the experiment was obtained from the Institutional Animal Ethics Committee (IAEC), (Ref No: KCP/IAEC/PCOL/PCEU/65/2020).

DATA AVAILABILITY STATEMENT

Authors state that all data is presented in the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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Nil

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REFERENCES

1. Manford M. Recent advances in epilepsy. J Neurol. 2017; 264(8):1811–1824.
2. Olkkola KT, Ahonen J. Midazolam and other benzodiazepines. Modern Anesthetics. 2008:335-360.
3. Cornett EM, Nemomsa MA, Turbeville B, Busby MA, Kaye JS, Kaye AJ, et al. Midazolam nasal spray to treat intermittent, stereotypic episodes of frequent seizure activity: pharmacology and clinical role, a comprehensive review. Health Psychol Res. 2022;10(5).
4. Erdő F, Bors LA, Farkas D, Bajza Á, Gizurarson S et al. Evaluation of intranasal delivery route of drug administration for brain targeting. Brain Res bulletin. 2018; 143:155-170.
5. Bonferoni MC, Rassu G, Gavini E, Sorrenti M, Catenacci L, Giunchedi P et al. Nose-to-brain delivery of antioxidants as a potential tool for the therapy of neurological diseases. Pharmaceutics. 2020; 12(12):1246.
6. Patel S, Koradia H, Parikh R. Design, and development of intranasal in situ gelling system of Midazolam hydrochloride using 32 full factorial design. J Drug Deliv Sci and Technol. 2015; 30:154-162.
7. Gholizadeh H, Cheng S, Pozzoli M, Messerotti E, Traini D, Young P, Kourmatzis A, Ong HX et al. Smart thermosensitive chitosan hydrogel for nasal delivery of ibuprofen to treat neurological disorders. Expert Opin Drug Deliv. 2019; 16 (4):453-466.
8. Kapoor M, Cloyd JC, Siegel RA. A review of intranasal formulations for the treatment of seizure emergencies. J Controlled Release. 2016; 237:147-159.
9. Su Y, Sun B, Gao X, Dong X, Fu L, Zhang Y, et al. Intranasal delivery of targeted nanoparticles loaded with miR-132 to brain for the treatment of neurodegenerative diseases. Front Pharmacol. 2020;11.
10. Zeeshan M, Mukhtar M, Ul Ain Q, Khan S, Ali H. Nanopharmaceuticals: A Boon to the Brain-Targeted Drug Delivery, Pharmaceutical Formulation Design - Recent Practices. IntechOpen; 2020.
11. Pal SL, Jana U, Manna PK, Mohanta GP, Manavalan R. Nanoparticle: An overview of preparation and characterization. J Applied Pharm Sci. 2011;(6):228-34.
12. Formica ML, Real DA, Picchio ML, Catlin E, Donnelly RF, Paredes AJ. On a highway to the brain: A review on nose-to-brain drug delivery using nanoparticles. Appl Mater Today. 2022; 29(101631):101631.
13. Islam SU, Shehzad A, Ahmed MB, Lee YS. Intranasal delivery of nanoformulations: A potential way of treatment for neurological disorders. Molecules. 2020;25(8):1929.
14. Sonawane RO, Bachhav Y, Tekade AR, Pardeshi CV. Nanoparticles for direct nose-to-brain drug delivery: Implications of targeting approaches. In: Direct Nose-to-Brain Drug Delivery. Elsevier. 2021: 157–168.
15. Nigam K, Kaur A, Tyagi A, Nematullah M, Khan F, Gabrani R, Dang S et al. Nose-to-brain delivery of lamotrigine-loaded PLGA nanoparticles. Drug Deliv Transl Res. 2019;9(5):879-890.
16. Kızılbey K. Optimization of rutin-loaded PLGA nanoparticles synthesized by single-emulsion solvent evaporation method. ACS Omega. 2019;4(1):555–562.
17. Joshi AS, Patel HS, Belgamwar VS, Agrawal A, Tekade AR. Solid lipid nanoparticles of ondansetron HCl for intranasal delivery: development, optimization and evaluation. J Mater Sci: Mater Med. 2012;23(9):2163-2175.
18. Sharaf NS, Shetta A, Elhalawani JE, Mamdouh W. Applying Box–Behnken Design for formulation and optimization of PLGA-coffee nanoparticles and detecting enhanced antioxidant and anticancer activities. Polymers (Basel). 2021; 14(1):144.
19. Wavikar PR, Vavia PR. Rivastigmine-loaded in situ gelling nanostructured lipid carriers for nose to brain delivery. J Liposome Res. 2015; 25(2):141-149.
20. Abdel-Bar HM, Abdel-Reheem AY, Awad GA, Mortada ND. Evaluation of brain targeting and mucosal integrity of nasally administrated nanostructured carriers of a CNS active drug, clonazepam. J Phar & Pharm Sci. 2013; 16(3):456-469.
21. Fahmy UA, Badr-Eldin SM, Ahmed OA, Aldawsari HM, Tima S, Asfour HZ, Al-Rabia MW, Negm AA, Sultan MH, Madkhali OA, Alhakamy NA et al. Intranasal niosomal in situ gel as a promising approach for enhancing flibanserin bioavailability and brain delivery: In vitro optimization and ex vivo/in vivo evaluation. Pharm. 2020;(6):485.
22. Bhavna, Md, S., Ali, M., Ali, R., Bhatnagar, A., Baboota, S., & Ali, J, et al. brain targeting of Donepezil nanosuspension intended for nose: In vitro and in vivo safety evaluation. Int J Bio Macromol 2014; 67:418–425.
23. Sherafudeen SP, Vasantha PV. Development and evaluation of in situ nasal gel formulations of loratadine. Res Pharm Sci. 2015; (6):466.
24. Deshkar SS, Jadhav MS, Shirolkar SV. Development of carbamazepine nanostructured lipid carrier loaded Thermosensitive gel for intranasal delivery. Adv Pharm Bull. 2020; 11(1):150–162.
25. Nogueira C, Lemos-Senna E, da Silva Vieira E, Sampaio TB, Mallmann MP, Oliveira MS, et al. B-caryophyllene cationic nanoemulsion for intranasal delivery and treatment of epilepsy: Development and in vivo evaluation of anticonvulsant activity. J Nanopart Res. 2023; 25(1).