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INVESTIGATION OF POTENTIAL EFFICACY OF NANOSPANLASTIC VESICULAR DRUG DELIVERY SYSTEM FOR TARGETING THE BRAIN: FORMULATION, CHARACTERIZATION, AND IN-VIVO STUDIES

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ABSTRACT
Background: Edaravone, a potent antioxidant, has limited brain bioavailability due to poor solubility and restricted permeability across the blood-brain barrier (BBB). Intranasal delivery offers a promising alternative for brain targeting by bypassing the BBB. **Objective:** To develop and evaluate a nanospanlastic-based in-situ nasal gel formulation of edaravone for enhanced brain delivery. **Methodology:** A Quality by Design (QbD) approach was employed to identify and optimize critical formulation variables using Plackett-Burman and Central Composite Design. The optimized nanospanlastics were incorporated into a gellan gum-based ion-activated in-situ nasal gel and characterized through in vitro, ex vivo, and in vivo studies. **Results and Discussion:** The optimized formulation exhibited a particle size of 213.4 nm, a drug entrapment efficiency of 67.59%, and rapid gelation upon contact with nasal fluid. In vitro diffusion showed over 80% drug release within 30 minutes, while ex vivo studies confirmed improved permeation (flux: 7.8067 µg/cm²/hr). Histopathology revealed no nasal mucosal irritation. Pharmacokinetic studies in rats demonstrated significantly enhanced brain and plasma exposure compared to the marketed edaravone injection, with higher Cmax (78.73 ng/mL), Tmax (121.2 min), and AUC. **Conclusion:** The developed nanospanlastic-based nasal gel offers a non-invasive, effective strategy for brain delivery of edaravone, with potential to improve therapeutic outcomes in neurological disorders.

INTRODUCTION

Strategies for targeting the central nervous system (CNS) face challenges in reaching the brain due to the presence of a selective, permeable membrane, known as the blood-brain barrier (BBB). Intranasal drug delivery system offers a promising alternative by exploiting the olfactory region, bypassing the BBB & enhancing drug transport to the brain. This approach not only improves the drug targeting but could also reduce the systemic side effects and avoid hepatic metabolism. This ensures a higher extent of drug availability in the brain and improves the efficiency of the drug product [1]. Edaravone

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(EDR), a potent antioxidant, plays a crucial role in maintaining oxidative balance by neutralizing oxygen-free radicals. Elevated oxidative stress leads to the nitration of tyrosine molecules. This contributes to neuronal loss, a key factor in the progression of neurological disorders. Hence, managing this oxidative stress is a vital strategy in preventing neuronal damage [2]. Nanoparticles (NPs) have emerged as versatile carriers exploited by researchers for the development of targeted drug delivery systems due to their elasticity and surface modification properties. NPs can be a promising approach for improving the efficiency of nasal formulation due to their excellent transmucosal permeability [3]. Among the various NPs, lipidic nanoparticles are the preferred carriers for solubilizing poorly water-soluble drugs due to their rapid uptake, biodegradability, and low toxicity.

Nanospanlastics (NSLs), vesicular carriers are primarily composed of non-ionic surfactant Span and an edge activator that enhances vesicular deformability and improves permeability across biological membranes [4,5]. They have demonstrated superiority over other vesicular carriers in terms of flexibility and stability. Spanlastics are more elastic and deformable than liposomes, while they are more stable and less irritant than transferosomes. Hence, they show effective penetration into the biological membrane. Despite these benefits, vesicular drug delivery systems face challenges, including optimizing vesicle size, drug loading, and controlled drug release. To address these challenges, this study employs a Quality by Design (QbD) approach, systematically identifying and optimizing critical quality attributes (CQAs) such as high drug encapsulation efficiency (>40%), small particle size (<200 nm), and prolonged stability (at 4°C). This study emphasizes the importance of optimizing parameters to ensure a consistent and high-quality drug product with the desired characteristics, while minimizing development costs and time. However, a systematic investigation of the influence of variance in the parameters on vesicle preparation remains limited. To bridge this gap, preliminary studies were performed to identify the most critical parameters affecting the drug entrapment and particle size. Subsequently, the Central Composite Design (CCD) was employed to evaluate the effects of various variable factors and their interactions on the desired outcome. Furthermore, the optimal formulation and process parameters were identified and validated for robustness and model accuracy through the application of the central composite design.

MATERIALS AND METHODS

Materials

Sorbitan-based non-ionic surfactants Span 60 & 40, Tween 80, and polyvinyl alcohol (PVA) were purchased from Molychem, Vadodara. Edaravone (EDR) was obtained as a gift sample from the Sun Pharma Advanced Research Company (SPARC). All the other reagents used were of an analytical grade.

Methods

Preparation of the EDR nanospanlastics

Spanlastic vesicles were prepared using the ethanol injection method [6]. Briefly, the aqueous phase containing the edge activator (Tween 80) was heated at 60°C on the magnetic stirrer. The organic phase consisted of chloroform and ethanol (9:1), based on the solubility studies of the drug and excipients. The non-ionic surfactant (Span 60) and EDR were solubilized in an organic solvent and injected at a constant rate into the aqueous phase, with continuous stirring maintained at 60°C for 30 minutes, followed by probe sonication (2 S 2 P) for 5 minutes. The resultant dispersion was kept refrigerated until further evaluation.

CHARACTERIZATION OF THE VESICLES

% Drug Entrapment efficiency (%EE)

Drug entrapment efficiency was calculated by mechanical dispersion followed by centrifugation. Vesicle dispersion was centrifuged in the ultra-centrifuge at 10,000 RPM for 30 min at 4 °C. Settled vesicles were mixed with 25 ml of methanol and shaken for 1 hr in the mechanical shaker for rupturing, followed by centrifugation in the ultra-centrifuge. From the supernatant, the entrapped drug was analyzed using a UV-spectrophotometer at λmax 283 nm, according to the following equation.

$$\% \, \boldsymbol{EE} = \frac{\boldsymbol{entrapped\ drug}}{\boldsymbol{total\ drug}} \boldsymbol{X\ 100}$$

Particle size and zeta potential measurement

Particle size and polydispersity index (PDI) of all the prepared formulations of spanlastic vesicles were determined by dynamic light scattering mechanism using Malvern Zetasizer. Samples were appropriately diluted with deionized water before analysis.

Experimental design

A Plackett-Burman design (PBD) was applied to screen the most significant process and formulation parameters affecting the characteristics of the vesicles. The type of non-ionic surfactant

(Span60 & 40), type of edge activators (Tween80 & PVA), hydration vol. (10ml & 20mL), hydration time (60min & 180min) & type of solvent (ethanol & chloroform: ethanol) were selected as independent variables. Design expert 11 software was used for the PBD studies, and based on the design output 12 diff. batches were prepared & analyzed for the CQAs. (Table 1)

Table 1: Formulation sets based on Plackett-Burman design

Batch No	X_1	X_2	X_3 (mL)	X_4 (min)	X_5	% EE	Particle size (nm)
PBD1	Span 60	Tween 80	20	180	Ethanol	65.93	325.6
PBD2	Span 40	Tween 80	10	180	Ethanol	40.21	123.1
PBD3	Span 60	PVA	10	60	Ethanol	45.93	93.5
PBD4	Span 60	Tween 80	10	60	Chloroform: Ethanol	55.39	145.7
PBD5	Span 60	Tween 80	20	60	Chloroform: Ethanol	49.12	165.6
PBD6	Span 60	PVA	20	60	Ethanol	50.32	541.8
PBD7	Span 60	PVA	20	60	Chloroform: Ethanol	42.46	654.4
PBD8	Span 60	Tween 80	20	180	Chloroform: Ethanol	62.31	653.7
PBD9	Span 60	PVA	10	180	Chloroform: Ethanol	40.19	107.3
PBD10	Span 60	Tween 80	10	60	Ethanol	39.51	256.6
PBD11	Span 60	PVA	10	180	Chloroform: Ethanol	44.39	432.4
PBD12	Span 60	PVA	20	180	Ethanol	44.92	345.5

Based on the screening studies, Span 60, Tween 80, and hydration volume were selected for further studies as they were the most influential factors using CCD. According to the CCD matrix, a total of 20 batches were generated, comprising 11 factorial points, 4 axial points, and 5 replicates for statistical assessment (Table 2).

Table 2: Formulation sets based on Central Composite design

Batch No	Conc. of Span 60(X_1)	Conc. of Tween 80(X2)	Hydration volume(X3)(mL)	% EE	Particle size(nm)
CCD 1	160	30	20	52.74	108.3
CCD 2	180	20	15	59.12	113.1
CCD 3	160	30	10	52.34	106.6
CCD 4	200	10	10	65.18	217.8
CCD 5	200	30	20	61.12	124.3
CCD 6	160	10	20	43.11	205.2
CCD 7	200	10	20	55.48	283.7
CCD 8	180	3.182	15	49.6	263.6
CCD 9	180	20	15	59.12	113.1
CCD 10	180	20	23.41	61.36	118.5
CCD 11	213.64	20	15	58.43	207.32
CCD 12	146.36	20	15	35.34	109.1
CCD 13	180	36.82	15	65.91	128.6
CCD 14	200	30	10	65.95	149.5
CCD 15	180	20	15	59.12	113.1
CCD 16	180	20	15	59.12	113.1
CCD 17	180	20	6.59	61.02	98.31
CCD 18	180	20	15	59.12	113.1
CCD 19	160	10	10	47.63	154.17
CCD 20	180	20	15	59.12	113.1

In-situ nasal formulation

The formulation of the in situ nasal gel system was performed by the reported method [7]. Briefly, gellan gum, an ion-sensitive polymer, was dispersed in hot water maintained at 80 °C. The solution of hydroxypropyl methylcellulose (HPMC) was added to it with constant stirring. The dispersion was left undisturbed at room temperature for 12 hrs to ensure complete solubilization of polymers. Finally, vesicle dispersion was added to the polymeric solution, along with humectant and preservative.

CHARACTERIZATION OF IN SITU GEL

Gelation time

Visual inspection was used to assess the gelation time of the formulation. A beaker containing a nasal formulation was placed on a magnetic stirrer at room temperature, and simulated nasal fluid (SNF) was added dropwise, triggering the sol-gel transition. The time taken for this transformation was recorded as the gelation time.

Mucoadhesive strength

Modified pan method was used to assess the mucoadhesive strength of the prepared in-situ gel using fresh nasal mucosa. The setup involved two 10 mL glass beakers. One beaker was affixed in an inverted position while the other was attached in place of the pan of the weighing balance. Both the beakers were separated by the nasal mucosa. 1 g of in situ gel was placed on the 7 cm^2 mucosal tissue, and both beakers were kept in contact for 2 minutes. In the second pan, the weight was increased continuously till both the beakers were detached [8].

The mucoadhesive strength was determined based on the applied detachment stress in dynes/cm^2.

$$\textit{Mucoadhesive strength} = \frac{\textit{Detachment weight } (m)}{\textit{Area exposed } (A)} \textit{ X Gravity } (g)$$

In-vitro diffusion study

The in vitro diffusion studies were carried out using a Franz diffusion cell with a preactivated 12-15 kDa (Himedia 150) dialysis bag. The dialysis bag with formulation equivalent to 30 mg of EDR was positioned between the receiver cells containing SNF and donor cells of the diffusion cell. The entire setup was maintained at 32 °C ± 2 °C with a rotation speed of 100rpm. At predeter- mined time intervals, 0.5 mL of the samples was withdrawn & analyzed at 243nm to determine drug diffusion per unit time.

Ex vivo nasal diffusion and permeation study

An ex vivo nasal diffusion and permeation study was conducted using a Franz diffusion assembly with goat nasal mucosa. The cartilage was carefully scraped off and separated from the nasal mucosa, which was thoroughly cleaned using saline phosphate buffer (pH 7.4).

The donor and receptor compartments were separated by the nasal mucosa and allowed to stabilize with SNF for 30 minutes before mounting the formulations. At predetermined intervals, 0.5 mL of the sample was withdrawn for analysis. To assess the ease of drug permeation through the nasal mucosa, evaluating parameters such as the permeability coefficient and permeability flux is essential.

These factors provide crucial insights into the drug's ability to traverse the nasal membrane effectively. The drug permeation per unit area of mucosa (µg/cm^2) and permeability flux (µg/cm^2.hr) were calculated using the following equation:

$$J\ (\textit{permability flux}) = \frac{\textit{amount of drug permeated from nasal mucosa}}{\textit{time}}$$

$$\textit{Permeability coeffeicent} = \frac{\textit{Permeability flux}}{\textit{total donar concentration}}$$

Histopathology

To evaluate the possible discomfort and irritation, histopathological evaluation of the 6-hour-treated goat nasal mucosa was conducted. After treatment, the tissue was immediately submerged in a phosphate buffer (pH 7.4) to maintain tissue integrity.

Pharmacokinetic activity

Two groups of Albino Wistar rats (n=5, 250-300 gm) of either sex were used. Group I received in-situ nasal formulation via the nasal route, and Group II received the marketed EDR injection via the i.v. route at dose of 3.75 mg/kg. At predetermined time intervals (15 min, 30 min, 60 min, 4 hrs, and 8 hrs), 0.5 mL of blood was extracted from retro-orbital plexus into precoated EDTA tube under mild anesthesia.

After blood collection, the animals were sacrificed to harvest brain tissues for further analysis. The study protocol was approved by the Krishna School of Pharmacy & Research, Krishna EDU Campus, KPGU, Vadodara-Mumbai NH#8, Varnama, Vadodara- 391243 (KSP/ IAEC/ 2023/ 03).

RESULT AND DISCUSSION

Screening of the most influential variables on the CQAs using PBD

Non-ionic surfactants having HLB values ranging between 3 and 8 are preferred for the formation of bilayer vesicles. Previous research suggests that surfactants with alkyl chain length between C12 and C18 are most suitable for achieving greater entrapment efficiency, as they demonstrate formation of more stable and robust vesicles [9]. Results of PBD demonstrated high drug entrapment with Span 60 compared to Span 40. This might be attributed to its C18 alkyl chain and 4.7 HLB values. Being non-ionic, Tween 80 aided the formation of spherical, narrow-range micellar vesicles. This could be a key factor for the formation of unilamellar vesicles [10]. Due to its HLB value of 15, Tween80 provided optimum interfacial stabilization compared to PVA, which directly influenced particle size & stabilized the dispersion against coalescence. With an increase in the hydration volume, a reduction in the viscosity of the dispersion medium occurs, facilitating drug diffusion from the organic layer to the aqueous layer & thereby improving drug entrapment. Lower viscosity facilitated the application of high shear stress to the prepared vesicles, resulting in a reduction in vesicle size.

Statistical analysis using the central composite design

For formula optimization, a central composite design was employed to determine the optimal combinations of influencing factors that would achieve the target responses. The findings from the PBD studies indicated that the nanospanlastics formulation was significantly influenced by the concentration of Span 60, Tween 80, and hydration volume. These were further optimized by CCD. Among the tested models, the quadratic model was found to be the most effective, outperforming both the linear and two-factor models (R^2 = 0.9381, at a 95% confidence level). Therefore, quadratic interaction and quadratic terms were selected to describe the impact of independent variables on formulation characteristics. The effects of the model term and associated p-values are presented in Table 3. Based on the obtained p-values, it was confirmed that the selected quadratic model effectively explains the relationship between the variables. Therefore, it is a suitable choice for optimizing and predicting formulation characterization.

Table 3: Statistics of model summary.

Parameters	Source	Degree of freedom	Sum of square	Mean of square	F value	p value
% EE	Model	9	1111.88	123.54	32.99	< 0.0001
	Residue	10	37.45	3.74		
	Lack of fit	5	37.45	7.49		
	Pure error					
Particle size	Model	9	58895.63	6543.96	67.33	< 0.0001
	Residue	10	971.95	97.19		
	Lack of fit	5	971.95	194.39		
	Pure error					

The regression equations for all the dependent variables were obtained as follows.

Entrapment efficiency

As the concentration of Span 60 increases, the drug entrapment increases. This is due to the miscibility of the lipophilic drug with Span 60, along with the formation of a concentric bilayer. In addition, the use of an edge activator improves the drug entrapment. This may be due to the presence of a long alkyl chain, which increases the lipophilic segment in the formed vesicle, thereby accommodating more drug into the vesicles.

$$\%\,EE = -387.196 + 4.444\,X_1 + 1.037\,X_2 + 0.608X_3 - 0.0049X_1X_2 - 0.013X_1X_3 + 0.025X_2X_3 - 0.011X_1^2 - 0.004X_2^2 + 0.033X_3^2$$

Particle size

A higher concentration of Span 60 shows increments in both particle size and viscosity. This may indicate the resistance to the applied shear stress of sonication, thereby reducing its efficiency. Similarly, with an increase in Tween 80 concentration, a reduction in particle size was observed. This might be due to the prevention of particle aggregation.

$$Particle\ size = +112.71 + 26.82\,X_1 - 43.88\,X_2 + 9.33X_3 - 10.40X_1X_2 - 1.5X_1X_3 - 17.55X_2X_3 + 18.47X_1^2 + 31.87X_2^2 + 0.8644.24X_3^2$$

For investigating the optimum combination and quadratic effect of the selected independent variables on the desirable dependent variables, three-dimensional plots were used. In figures 1 (a) and (b), the influence of the amount of span 60 and Tween 80, along with their interaction, on particle size & % drug entrapment was graphically represented through 3D plots.

Validation of the model

Using Design Expert software and desirability analysis, an overlay plot was generated (yellow region, Figure 1-C) by applying constraints of a minimum particle size and 60-65% drug entrapment. The checkpoint batches indicated by the overlay plot were formulated and subsequently evaluated. The difference between the observed and predicted results was found to be less than 5%, confirming the validity of the selected model for further studies.

Based on the studies, the optimized batch was formulated using 184 mg Span 60, 30 mg Tween 80, and a hydration volume of 15 ml.

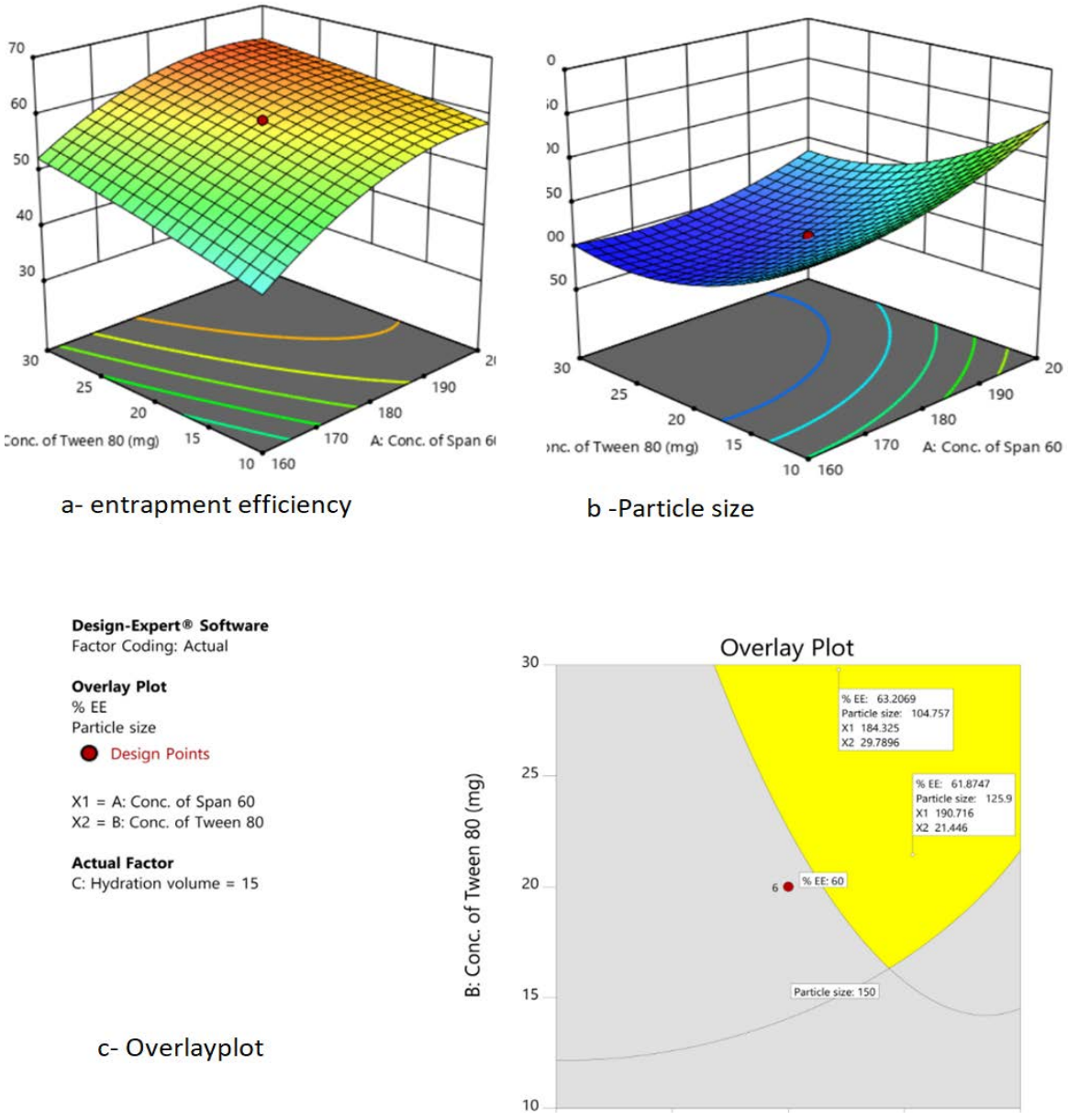

Figure 1: Response surface methodology showing the influence of concentration of Span 60 and Tween 80 on (a) %EE; (b) Particle size; (c) Overlay plot

CHARACTERIZATION OF NANOSPANLASTICS-LOADED NASAL IN SITU GEL

Mean Particle size and % EE

The mean particle size was found to be 213.4 nm with a polydispersity index of 0.517. This formulation showed 67.59±4.32% encapsulation of the drug.

Gelation time

Gellan gum undergoes a phase transition in the presence of cations found in body fluids through physical crosslinking, forming both covalent and non-covalent bonds [11,12]. The results revealed a gelation time of approximately 45-50 seconds for a small volume of SNF.

Mucoadhesive strength

In the presence of cation-rich SNF, an ion-sensitive nasal formulation formed an instant 3D network, exhibiting adhesion to the nasal cavity. This network is a key factor in nasal mucoadhesion and can prevent the drainage of the formulation from the nasal cavity [13]. This prolongs retention in the nose, allowing for maximum absorption from the olfactory region. The mucoadhesion force for the nasal formulation was observed to be 1971 dynes/cm^2.

In-vitro diffusion study

For the in vitro drug diffusion study, the Franz diffusion method was employed using SNF pH 5.5 as the dissolution fluid. As per Figure 2 (A), more than 80% of the drug was diffused from the EDR-NSL within 30 minutes, while the in-situ nasal formulation demonstrated a diffusion time of 60 minutes. The maximum regression coefficient values of plain dispersion (0.9561) and in-situ gelling system (0.944) for the Higuchi kinetics model were observed in release kinetics studies. Gellan gum and HPMC enhanced the entanglement of the drug in the formed porous gel, resulting in high gel strength and prolonged drug release [14]. The reason behind sustained and controlled drug release was the presence of the drug in the core of the concentric bilayer and deep into the vesicles [15].

Ex vivo nasal diffusion and permeation study

The permeation of drugs through the nasal mucosa is considered a significant parameter for understanding drug release from biological membranes. Goat nasal mucosa is a suitable choice for studying ex vivo permeation due to its similarity to human mucosa in terms of metabolism, transport mechanisms, and electrical impulse resistance [16]. Figure 2(B) indicates that more than 80% of the drug is permeated within two hours. Permeation flux and permeation coefficient were 7.8067 µg/cm2.hr and 23.015 µg/cm², respectively. Permeation enhancement could be attributed to the combined effects of factors such as the nano-sized range and the presence of a stabilizer, which can impart elasticity to the vesicles and facilitate penetration by temporarily destabilizing biological membranes.

Histopathology

The nasal irritation and toxicity of the nasal formulation were studied through histopathological examination of the nasal mucosa. The results indicated that in the untreated tissue, intact epithelium with columnar cells was observed, while normal mucosa lined by pseudostartified ciliated columnar epithelium was seen in the treated nasal mucosa (Figure 3). This finding was evidence for the absence of interaction between biological membrane and the formulation. This may be attributed to the biocompatibility profile of the non-ionic surfactant.

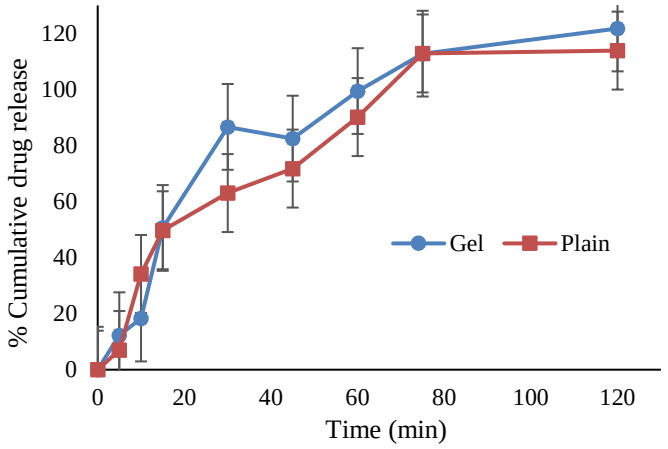

Figure 2A: In-vitro diffusion study

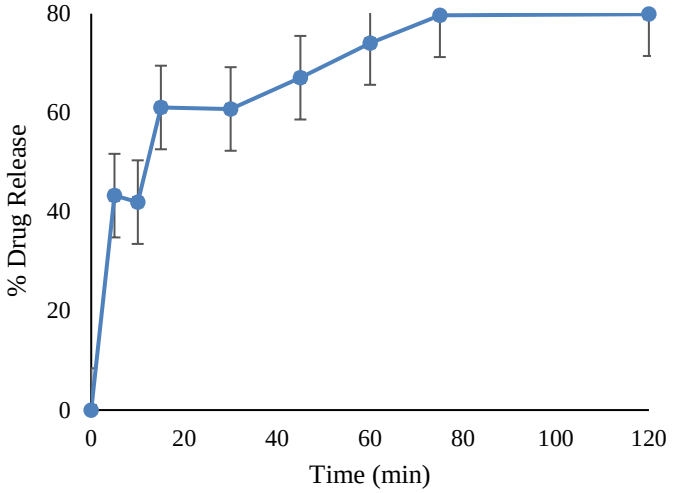

Figure 2 B: Ex-vivo drug diffusion study

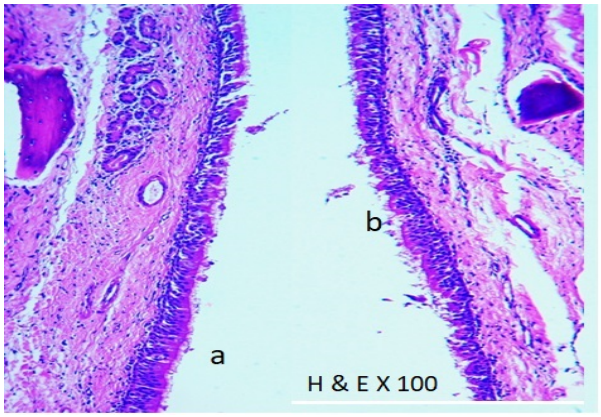

Figure 3: Histopathology study on goat nasal mucosa:(a) Control; (b) Treated

Pharmacokinetic activity

A pharmacokinetic study was performed with respect to the marketed formulation (Edastar injection, Lupin Ltd). The drug plasma concentration versus time profile and EDR biodistribution in the brain for the nasal in-situ gelling system are shown in Figure 4. Figure 5 shows the comparative studies of nasal formulation with marketed formulations. The results depicted in the figure confirmed the equivalence of efficiency of the prepared formulation with the marketed injection. The various pharmacokinetic parameters were calculated using PK solver software and shown in Table 4.

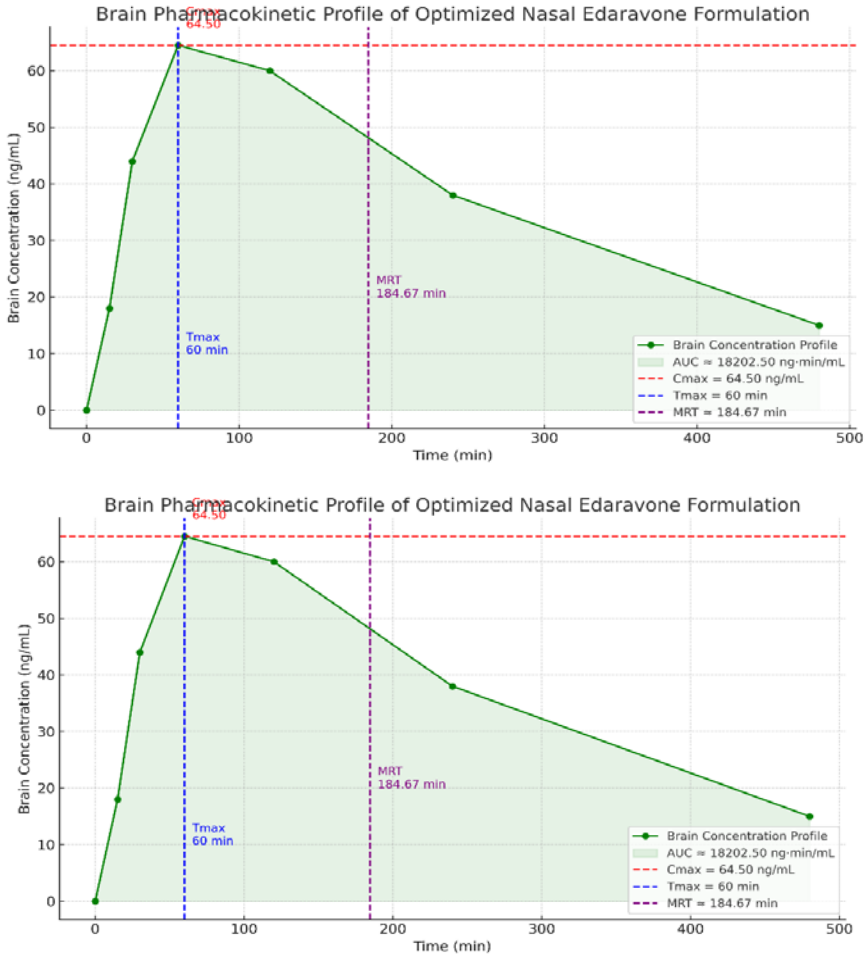

Figure 4: Pharmacokinetics studies of nasal formulation: (a) Plasma Concentration; (b) Brain Concentration

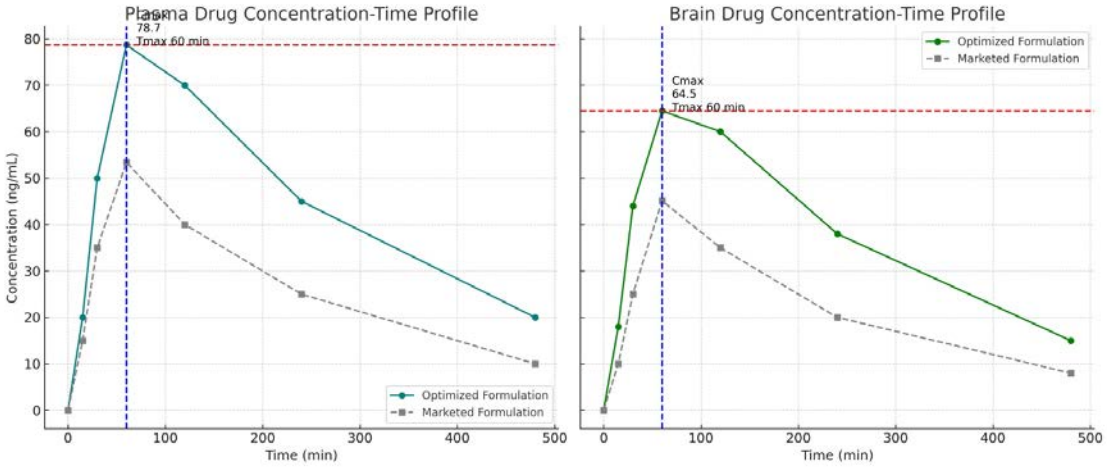

Figure 5: Plasma drug profile and brain distribution of the marketed formulation and the nasal formulation

Table 4: Pharmacokinetic parameters for optimized batch and marketed formulation

Pharmacokinetic parameter	EDR- nanospanlastics loaded nasal in-situ gel		Marketed formulation	
	Plasma	**Brain**	**Plasma**	**Brain**
T_{max} (min)	121.2040229	89.25511017	33.894	55.425
C_{max} (ng/ml)	78.7363691	64.50432655	53.416	45.137
AUC_{total} (ng/ml*min)	23501.96386	19194.99952	11507.40	9837.54
MRT (min)	242.8094898	299.5235909	213.010	192.65

DISCUSSION

The present study successfully developed and evaluated a nanospanlastic-based in-situ nasal gel formulation of edaravone aimed at enhancing brain delivery. By applying Quality by Design (QbD) principles, specifically Plackett–Burman and Central Composite Design (CCD), critical formulation and process parameters were identified and optimized. Span 60, Tween 80, and hydration volume emerged as the most influential variables affecting key quality attributes such as entrapment efficiency and particle size. The optimized formulation, incorporating 184 mg Span 60, 30 mg Tween 80, and a hydration volume of 15 mL, exhibited favorable physicochemical characteristics, including a particle size of 213.4 nm, a polydispersity index of 0.517, and a drug entrapment efficiency of 67.59%.

These results underscore the ability of nanospanlastics to encapsulate poorly soluble drugs efficiently while maintaining vesicle stability. When incorporated into a gellan gum and HPMC-based in-situ gel, the formulation exhibited rapid gelation upon contact with simulated nasal fluid, taking approximately 45–50 seconds. The mucoadhesive strength of 1971 dynes/cm² indicates adequate nasal retention, which is critical for enhancing drug residence time in the nasal cavity and promoting transport across the olfactory region. Although slightly below the typical threshold of 2000 dynes/cm² for optimal adhesion, the results remain promising, especially considering the biocompatibility of the selected polymers.

In vitro and ex vivo studies further confirmed the potential of this delivery system. Over 80% of drug diffusion was achieved within 30 minutes in vitro, while the ex vivo goat nasal mucosa model revealed efficient drug permeation with a flux of 7.8067 µg/cm²/hr. These findings can be attributed to the nanoscale size of the vesicles, the flexibility imparted by Tween 80, and the biocompatibility of Span 60. Importantly, histopathological analysis of the treated nasal mucosa showed intact epithelial integrity, indicating minimal to no irritation from the formulation. Span 60 is biocompatible & non-irritant to the mucosal surface. At the same time, Tween80 imparts fluidity to the mucosal epithelium, which might be the reason for the negligible variation in the nasal mucosal cells.

Pharmacokinetic evaluation revealed that the in-situ nasal gel achieved a higher C_max and prolonged T_max compared to the marketed intravenous formulation of edaravone. Brain distribution profiles supported enhanced nose-to-brain delivery, suggesting successful bypass of the blood–brain barrier via the olfactory route. Despite these pharmacokinetic improvements, a statistical comparison using a paired t-test revealed no significant difference in drug bioavailability between the test and marketed formulations ($p > 0.05$), confirming equivalence in systemic exposure. Collectively, the study demonstrates that nanospanlastic vesicles integrated into an in situ nasal gel system offer a promising non-invasive strategy for the effective brain targeting of edaravone. The design of such a formulation addresses not only the drug's solubility and permeability challenges but also ensures patient compliance and reduces systemic side effects.

CONCLUSION

The present study demonstrates the successful development and evaluation of a nanospanlastic-based in-situ nasal gel formulation of edaravone for targeted brain delivery. Employing a systematic Quality by Design (QbD) approach, critical formulation variables Span 60, Tween 80, and hydration volume were identified and optimized using Plackett–Burman and Central Composite Designs.

The optimized nanovesicular system exhibited desirable physicochemical characteristics, high drug entrapment, and a nanoscale particle size suitable for nasal administration.

Incorporation into an ion-sensitive in-situ gel provided rapid gelation, strong mucoadhesive properties, and sustained drug release, enhancing nasal residence time and facilitating effective nose-to-brain transport. The formulation demonstrated superior ex vivo permeation and favorable pharmacokinetic parameters, including prolonged brain residence and enhanced drug bioavailability, comparable to that of the marketed intravenous formulation. Histopathological evaluation confirmed the safety and biocompatibility of the formulation on nasal tissues.

Overall, this novel nasal delivery platform offers a non-invasive, patient-friendly alternative to conventional edaravone therapy, with potential for improved therapeutic outcomes in neurodegenerative disorders. The study provides a strong foundation for further clinical investigation and translational development of nasal nanovesicular drug delivery systems for CNS targeting.

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FINANCIAL ASSISTANCE
NIL

CONFLICT OF INTEREST
The authors declare no conflict of interest.

AUTHOR CONTRIBUTION
Prachi Pandey analyzed and interpreted the experimental design data related to the work. Ashwini Patel performed the studies in the laboratory, recorded observations, and contributed to drafting the manuscript. All authors read and approved the final manuscript.

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