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RESEARCH

Development and Evaluation of Nasal Microsphere Formulations for Enhanced Nose-to-Brain

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ABSTRACT

This study developed and evaluated lorazepam-loaded nasal microspheres using carbopol 934 and chitosan polymers to enhance nose-to-brain drug delivery for epilepsy treatment. Microspheres were formulated via spray-drying with particle sizes ideal for nasal administration (5–50 µm) and characterized for particle size, yield, drug content, and release profiles. Analytical techniques, including IR spectroscopy, DSC, and FESEM, confirmed drug stability, polymer compatibility, and morphology. Drug release studies showed high release percentages (93.59% for LCH3 and 93.16% for LC3), with quasi-Fickian diffusion kinetics. In vivo studies in rats demonstrated significantly higher brain drug concentrations for the optimized formulations, LCH3 (143.07%) and LC3 (150.70%), compared to pure lorazepam. These findings highlight the potential of nasal microspheres as an effective and patient-friendly approach for rapid brain-targeted drug delivery in epilepsy management.

KEYWORDS: Lorazepam, nasal microspheres, nose-to-brain delivery, epilepsy, spray-drying

INTRODUCTION:

Epilepsy, a chronic neurological disorder characterized by recurrent seizures, requires effective drug delivery strategies to manage its symptoms and prevent future episodes.(1)(2) Conventional drug delivery systems, such as oral tablets and injectable forms, often suffer from limitations like first-pass metabolism, systemic side effects, and delayed onset of action.(3) These challenges necessitate innovative approaches to achieve faster and targeted delivery of antiepileptic drugs.(4)

The nasal route offers a promising alternative for drug delivery, especially for central nervous system (CNS) disorders.(5) Due to the absence of the blood-brain barrier in the olfactory region, this route enables direct access of drugs to the brain, bypassing systemic circulation. (6) Microsphere formulations, with their ability to control drug release and enhance mucosal adhesion, provide an ideal platform for developing nasal drug delivery systems.(7)

This research focuses on the development and characterization of spray-dried lorazepam-loaded microspheres using chitosan and carbopol 934 polymers for nose-to-brain delivery in epilepsy management. The study investigates key formulation parameters, including particle size, drug entrapment efficiency, release kinetics, and in vivo drug distribution to the brain. By employing advanced analytical techniques such as infrared spectroscopy, differential scanning calorimetry (DSC), field emission scanning electron microscopy (FESEM), and ex vivo permeability studies, this work aims to establish the efficacy of these microspheres in delivering lorazepam directly to the brain.

The outcomes of this study highlight the potential of nasal microsphere formulations as a patient-friendly, efficient, and non-invasive alternative to conventional therapies for epilepsy, paving the way for improved clinical outcomes and enhanced patient compliance.

EXPERIMENTAL

Materials and reagents

The supplier of Lorazepam was Lab India Ltd. India

Table 1. Formulation Design for lorazepam batches

Polymer	Chitosan				Carbopol 934			
Drug	Lorazepam				Lorazepam			
Formulation code	LCH1	LCH2	LCH3	LCH4	LC1	LC2	LC3	LC4
Drug-Polymer ratio	0.5:1	1:1	1:1.5	1:0.5	0.5:1	1:1	1:1.5	1:0.5
Drug (mg)	500	1000	1000	1000	500	1000	1000	1000
Polymer (mg)	1000	1000	1500	500	1000	1000	1500	500

Sea Foods in Cochin was the source of the chitosan. Ethanol was acquired from Loba Chemie Pvt Ltd, HPLC grade methanol was acquired from Merck Pvt Ltd, Mumbai, and carbopol 934 was acquired from Lab India Ltd. Analytical grade reagents were the remaining ones utilized.

Formulations

The following ratios of lorazepam to the polymers chitosan and carbolpol 934 were used to construct the formations, and each batch was given a distinct formulation code

Preparation of spray-dried microspheres

The spray-drying technique was employed to develop the formulations. A spray dryer (Model SPD-D-111) from Techno Search Instruments was utilized to prepare batches with different drug-polymer ratios. The flow rate for all batches was maintained at a constant 1 ml/min, while the temperature was controlled within a range of 70°C to 100°C. Suspensions containing the drug and polymer were prepared using a solvent mixture of water and ethanol in a 1:1 ratio. The nozzle was adjusted to achieve a particle size distribution between 5µm and 50µm. (8)(9)(10)

Characterisation of microspheres

Appearance

Each batch of the formulation was evaluated based on key parameters such as color, and the results were documented for the different batches.(11)

Particle size analysis

Each formulation batch was analyzed for particle size using optical microscopy, and the average particle size was determined for all batches.(12)

Percentage yield

The percentage yield after spray drying was determined for all formulations.

Drug content determinations

Using UV spectroscopy on a Shimadzu UV-vis spectrophotometer model UV-3600i, the drug content was determined. Methanol was used to dissolve the lorazepam formulations, and distilled water was used to create dilutions. At λ max 228 nm, the formulation measurements were obtained. A calibration curve of lorazepam in the lambert bear range of 1–10 µg/ml concentration was used to determine the drug content. (8)

Infrared spectroscopy

The Bruker alpha ATR equipment was used to perform infrared spectroscopy. A sample of 1 mg was obtained for examination from each formulation batch. IR spectroscopy was investigated using the chosen compositions LCH3 and LC2.

Differential scanning calorimetry

The Mettler Toledo instrument was used for DSC. An aluminum sample holder with a temperature range of 25° C to 300°C was used for the analysis. Differential Scanning Calorimetry analysis was performed on the chosen formulations LCH2 and LC3. (13)

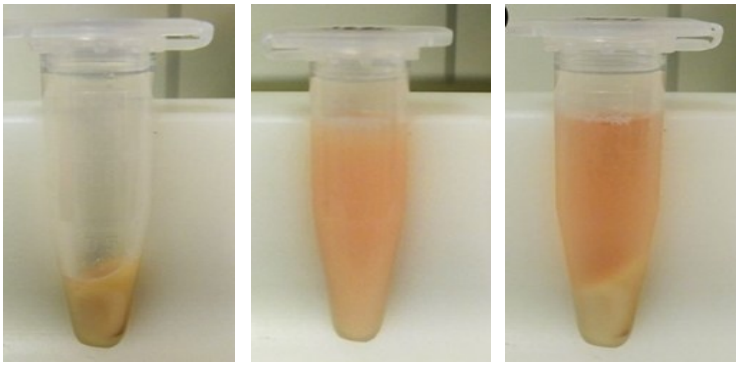

Fig. No. 1 Brain homogenate samples

Table 2. Preliminary Characterization of lorazepam batches

Batch	LCH1	LCH2	LCH3	LCH4	LC1	LC2	LC3	LC4
Particle size (µm)	25-39	27-46	22-48	29-48	23-46	22-37	22-46	19-37
Mean size (µm)	27.4	31.5	26.9	20.9	32.8	36.3	28.6	37.4
% yield	69.34	66.58	70.15	68.45	62.54	72.59	63.59	69.41
Drug content	92.35	97.52	95.64	91.37	93.87	96.11	98.05	93.24

Field emission scanning electron microscopy

Using the FEI Nova NanoSEM 450, FESEM was used to investigate the morphology and particle size of the microparticles. The resolution of the device was really great. Prior to being attached on carbon conductive adhesive tape on the sample holder, the 1 mg sample was first coated with gold particles. To observe the size and shape of the microparticles, the analysis was conducted at different resolutions.(14)

***Ex Vivo* permeability study**

Permeability analysis was conducted using Franz's

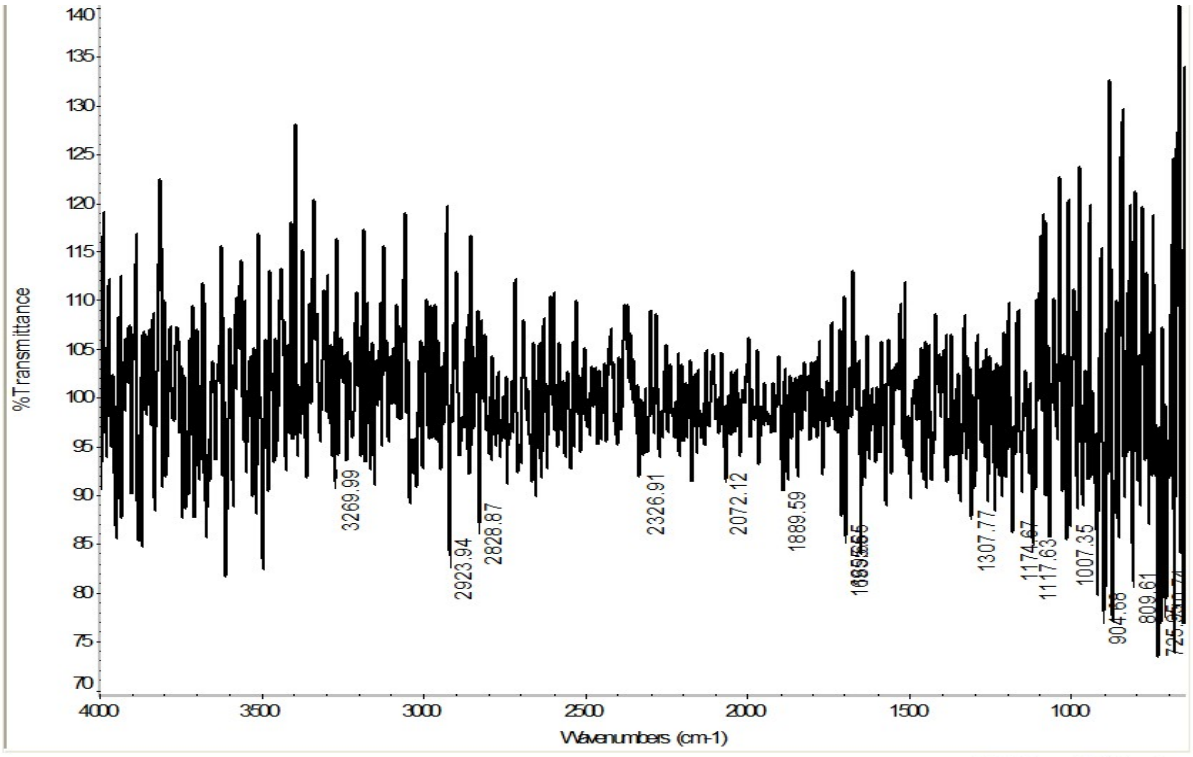

Fig. No. 2. Infrared Spectroscopy of LCH3 formulation

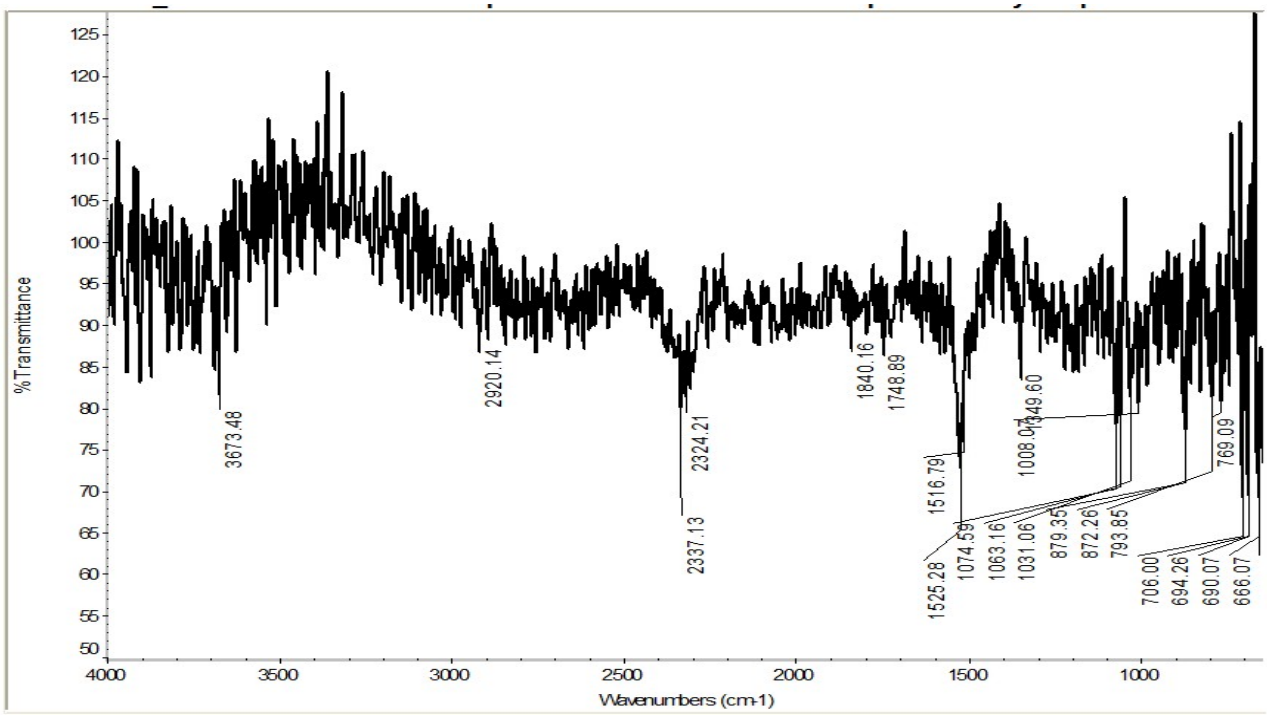

Fig. No. 3 Infrared Spectroscopy of LC2 formulation

diffusion cell since it is a crucial assessment to support medication absorption through the nasal membrane. A fifty milliliter Franz's diffusion cell was used, and fresh goat nasal mucosa from the wet market was used for the membrane. For additional diffusion research, the two best formulations of each polymer-chitosan and carbopol 934 were selected.(15)

In the receptor compartment, potassium dihydrogen phosphate and dipotassium hydrogen phosphate were used to provide phosphate buffer with a pH of 6.5 as a solvent. From each formulation, a sample equal to 4 mg of the medication lorazepam was extracted and applied to the donor compartment on the goat's nasal mucosal membrane. Following sample placement, the diffusion study was started, and at intervals of 5, 10, 15, 30, 45, 60, and 90 minutes, 1 ml aliquots were obtained from each sample. A UV spectrophotometer was used to evaluate the diluted samples, using a wavelength of 228 nm as the λ max.(16)

***In Vivo* studies**

1. Ethical statement

The in vivo studies were performed as per the CPCSEA guidelines. The animal study protocol was duly approved by the CPCSEA Approved drug testing Laboratory with registration 1410/c/11/CPCSEA. The CPCSEA/IAEC number given for the *In Vivo* study is CPCSEA/IAEC/0220/156

2. Albino rat model method: Inbred Albino Rats were used for the study. The animals were housed in individually ventilated cages under controlled noise and temperature conditions. They were provided with a standard pellet diet and had free access to water.

Test samples were prepared by diluting in PBS to a concentration of 50 mg/ml. A 20 µl solution was administered into each nostril of the rats and left for 15 minutes. The animals were then sacrificed, and their brains were carefully dissected and kept on ice. The brains were homogenized in PBS at a concentration of 100 mg/ml using a Remi homogenizer. The homogenized samples were centrifuged at 5000 rpm, and the supernatant was collected. Precipitation of the supernatants was carried out using methanol (500 µl homogenate + 50 µl methanol). The samples were further centrifuged, and the resulting supernatant was used for HPLC analysis.

3 HPLC analysis

The Shimadzu HPLC type LC-2050 was used to perform the HPLC analysis. For the analysis, a C-18 column with dimensions of 250 mm in length, 4.6 mm in internal diameter, and 5 µm particle sizes was employed. The flow rate of 1

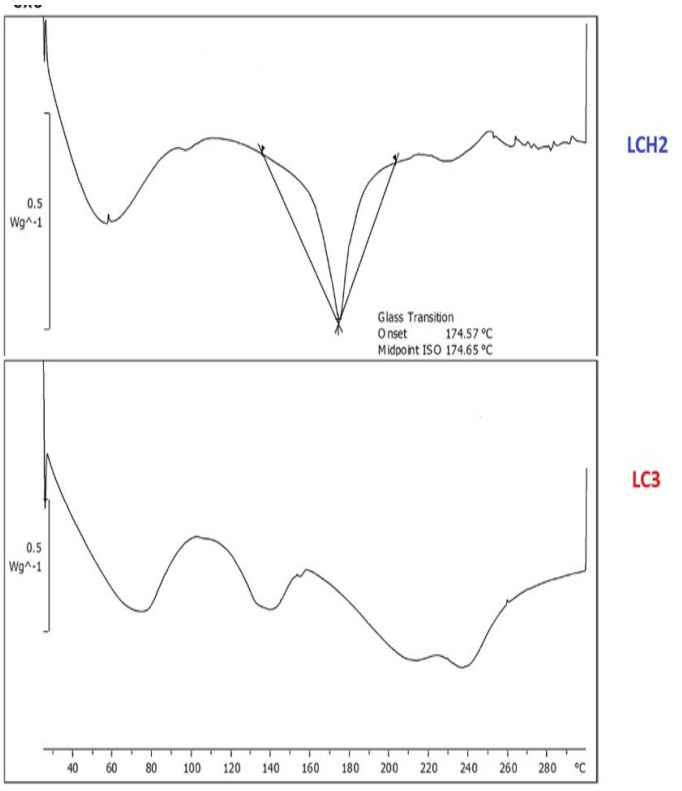

Fig. No. 4. Differential scanning calorimetry of LCH2 & LC3 formulations

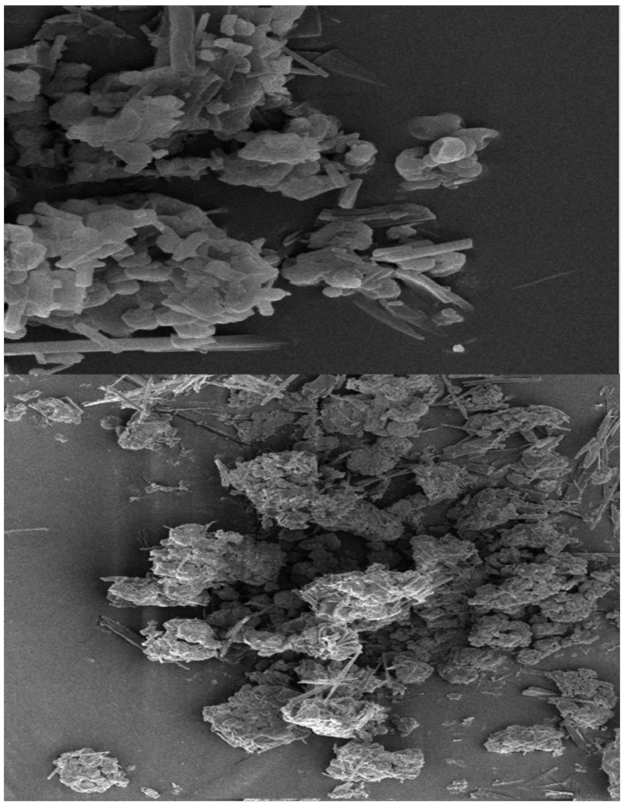

Fig. No. 5. FESEM of LCH3 formulation A) 5-50µm particles B) Drug embedded in the polymer

milliliter per minute at room temperature for lorazepam-methanol:water 65:35 is observed using UV detection at wavelengths of 255 and 230 nm.

RESULTS

Appearance, particle size, % yield and drug content:

While the batches containing lorazepam and Carbopol 934 appeared white, those formulated with chitosan exhibited a yellowish tint. The particle size for Carbopol 934 formulations ranged from 22 µm to 46 µm, whereas chitosan formulations had a particle size range of 22 µm to 48 µm. The average particle size for chitosan formulations varied between 20.9 µm and 31.5 µm, while for Carbopol 934, it ranged from 28.6 µm to 37.4 µm. The yield varied between 66.58% and 70.15% for chitosan formulations and between 62.54% and 72.59% for carbopol 934. UV spectrophotometry was also used to determine the drug concentration, which varied between 91.37% and 97.52% for chitosan formulations and between 93.24% and 98.05% for carbopol 934 formulations. (Table No. 2)

Infrared Spectroscopy:

IR spectroscopy was performed on the formulations LCH3 and LC2. The majority of the characteristic peaks corresponding to the functional groups of lorazepam remained intact in both spectra, which were found to be consistent. In the LCH3 formulation, peaks were observed at 3254 cm^{-1}, 3201 cm^{-1}, 1704 cm^{-1}, 1662 cm^{-1}, 1630 cm^{-1}, 1562 cm^{-1}, and 1555 cm^{-1}. Meanwhile, the LC2 formulation exhibited peaks at 3439 cm^{-1}, 3380

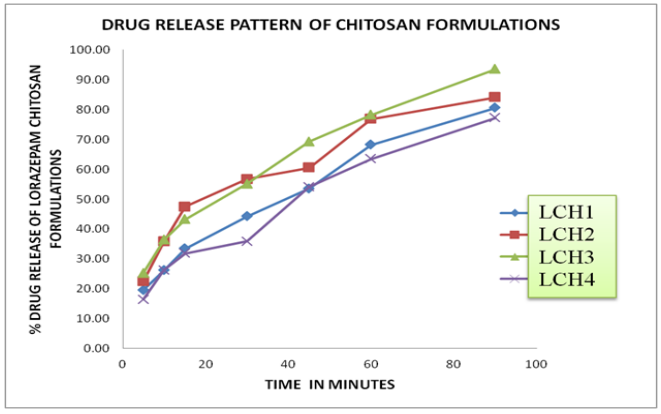

Fig. No. 6 Drug release pattern of lorazepam

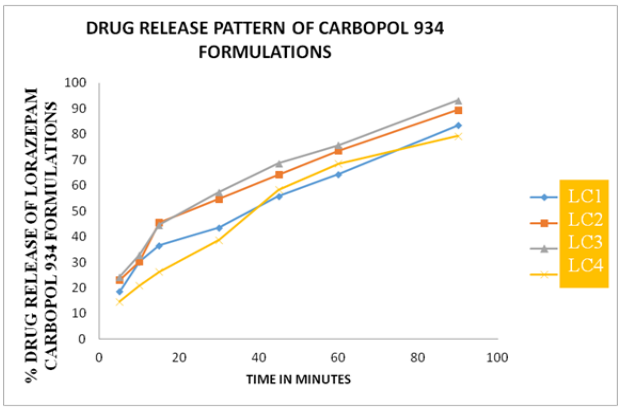

Fig. No. 7. Drug release pattern of lorazepam

Table no. 3 Release Kinetics for all formulations

Formulation	Release curve equation	R^2
LCH1	y = 0.7166x + 20.43	0.9751
LCH2	y = 0.6687x + 30.523	0.8947
LCH3	y = 0.776x + 29.051	0.9619
LCH4	y = 0.6989x + 18.181	0.9665
LC1	y = 0.7078x + 21.685	0.9745
LC2	y = 0.7419x + 27.37	0.9409
LC3	y = 0.7758x + 28.369	0.9491
LC4	y = 0.7976x + 14.727	0.9562

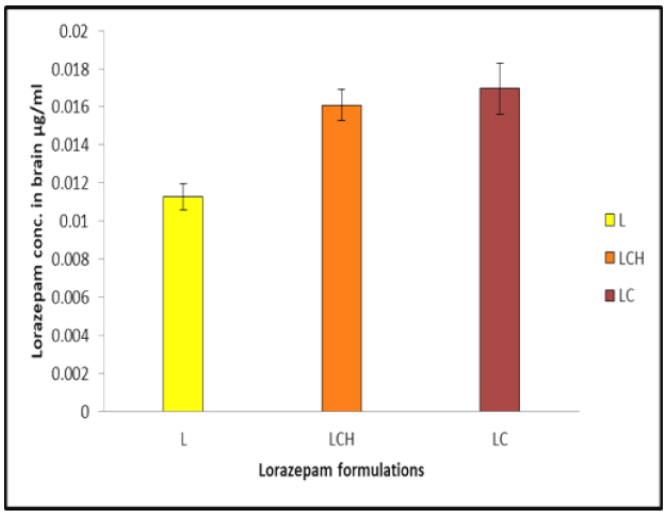

Fig. No. 8 Concentrations of lorazepam in brain

Table No. 4 HPLC analysis for lorazepam formulations

Formulation Code	1	2	3	4	5	Average	S.D.
L	0.01038	0.0109	0.01208	0.01177	0.01123	0.01126	0.00068
LCH3	0.01547	0.0163	0.01744	0.01589	0.01541	0.01609	0.00083
LC3	0.01646	0.0158	0.01634	0.01929	0.01685	0.01695	0.00135

cm^{-1}, 3297 cm^{-1}, 1696 cm^{-1}, 1618 cm^{-1}, 1538 cm^{-1}, 1267 cm^{-1}, and 1055 cm^{-1}.

Differential scanning calorimetry:

Differential scanning calorimetry was used to analyze the LCH2 and LC3 formulations. The graphs for both formulations displayed distinct endothermic peaks corresponding to lorazepam. A broad initial peak was observed between 60°C and 80° C in both LCH2 and LC3, with greater prominence in the Carbopol-based LC3 formulation. In LCH2, which contains chitosan, a characteristic wide peak at 174°C indicated the melting point of lorazepam. However, in the Carbopol formulation LC3, no distinct peak for the melting point of lorazepam was observed.

Field Emission Scanning Electron Microscopy:

To examine the size and external morphology of the microparticles, analysis was conducted at varying resolutions. The observed particle size ranged from 5 to 50 µm, which is suitable for nasal drug delivery. Particles smaller than 5 µm may reach the lungs, while those exceeding 50 µm may not adhere effectively to the mucosal surface. Additionally, the particles exhibited an ellipsoid or spherical shape. At higher resolutions, the drug was visibly embedded within the polymer matrix.

***Ex Vivo* permeability study**: To critically assess drug absorption through the nasal membrane, a permeability study was conducted using Franz's diffusion cell. Formulations containing Carbopol 934 and chitosan were utilized for this investigation. The drug release percentage from each formulation was determined by measuring absorbance with a UV spectrophotometer. Additionally, release kinetic equations were analyzed to identify potential drug release models. The LCH3 formulation exhibited the highest drug release percentage at 93.59 ± 0.32%, while other chitosan-based batches showed drug release ranging from 77.28% to 93.59%. For Carbopol 934 formulations, drug release varied between 79.26% and 93.16%, with the highest release of 93.16 ± 0.16% observed in the LC3 formulation.

When analyzing drug-release kinetics using the Korsmeyer-Peppas model, all chitosan and Carbopol 934 formulations exhibited regression values of less than 1.

***In Vivo* Studies:**

For the study, inbred albino rats were employed. After centrifuging the homogenized brains in PBS, the supernatant was collected. After centrifuging, the supernatant was utilized for HPLC analysis.

To calibrate lorazepam on HPLC at room temperature, a 65:35 methanol and water mixture was used. A 1.0 ml/min flow rate was used. For further calculations, the obtained AUC of 24568 at 0.01 µg/ml was used as a benchmark. The HPLC method was used to assess the AUC for the enhanced formulations LCH3 and LC3.

HPLC analysis was used to determine drug concentrations in the brain homogenate by analyzing the supernatants obtained after centrifugation. Single-point detection was performed 15 minutes post-administration to measure drug concentrations in the brain tissue homogenate. This analysis was conducted to investigate the rapid onset of action via this delivery route. Additionally, calculations were completed within a 20-minute timeframe, aligning with the commonly recorded mucociliary clearance time.

The in vivo analysis measured lorazepam concentrations in brain tissue for both the pure drug (used as a reference) and the selected formulations, LCH3 and LC3. The chitosan-based formulation, LCH3, exhibited a 143.07±6.01% higher drug concentration in brain tissue compared to the pure drug. Similarly, the Carbopol 934 formulation, LC3, showed an increase of up to 150.70±9.99% in drug concentration compared to pure lorazepam.

Discussion: Various factors, including stress, are leading to a rise in central nervous system (CNS) disorders such as bipolar disorder, epilepsy, Alzheimer's disease, and migraines. The treatment for these conditions primarily involves conventional dosage forms like tablets, capsules, and, in some cases, parenteral administration. Traditional dosage forms face limitations such as first-pass metabolism, protein binding, and poor brain penetration, necessitating the development of advanced drug delivery systems. Since the olfactory region lacks a blood-brain barrier, this study focused on utilizing the nose-to-brain delivery route. Compared to the parenteral method, nasal drug delivery offers the advantage of faster drug transport to brain tissue.

The produced spray-dried microspheres were appropriate for delivery into the nasal cavity since their particle sizes ranged from 5 to 50 micrometres. Most formulations had promising percentage yields and drug content, and the formulations with the best findings were picked for further testing.

As shown in Figures 2 and 3 from the infrared spectroscopy analysis, the lorazepam peaks remained unchanged or

were only slightly shifted by the polymers, with no significant changes in the drug's properties. This indicates that there is no apparent drug-polymer interaction within the formulations.

Differential scanning calorimetry (DSC) was used to analyze LCH2 and LC3 to assess the physical state of the drug within the microspheres. The results, shown in Figure 4, revealed a prominent endothermic peak at 174°C due to the drug's melting. The absence of this peak in the DSC thermogram for lorazepam-loaded microspheres suggests that the drug was molecularly dispersed within the microspheres.

Field emission scanning electron microscopy images of the LCH3 formulation, as shown in Figure 4, revealed a typical spherical or ellipsoid shape with no presence of free drug. At higher resolutions, the drug was observed to be embedded within the polymer network, except for some needle-like structures.

The regression values for all chitosan and Carbopol 934 formulations were below 1. Analysis of drug-release kinetics using the Korsmeyer-Peppas model showed that the "n" value for the formulations exceeded 0.5, indicating a Fickian-type release pattern. This release behavior was attributed to the similarity between the solvent diffusion time and polymer relaxation time.

The study demonstrated that spray-dried microsphere formulations enhance lorazepam delivery from the nose to the brain, showing higher drug concentrations in brain tissue compared to the pure drug. The LCH3 and LC3 formulations increased drug levels by 143.07±6.01% and 150.70±9.99%, respectively. Field emission scanning electron microscopy confirmed proper drug entrapment and morphology, while optical microscopy measured particle sizes between 5–50 µm, ideal for nasal delivery. Ex vivo permeability studies showed high drug release rates (LCH3: 93.59 ± 0.32%, LC3: 93.16 ± 0.16%), aligning with the quasi-Fickian diffusion model. The promising in vivo results suggest these formulations could improve epilepsy treatment. Future research will focus on developing suitable nasal delivery devices for improved patient compliance.

CONCLUSION

This study successfully developed and evaluated lorazepam-loaded nasal microspheres using carbopol 934 and chitosan polymers to enhance nose-to-brain drug delivery for epilepsy treatment. The formulations demonstrated optimal particle size, high drug content, and favorable drug release profiles, with release kinetics following a quasi-Fickian diffusion model. Advanced characterization techniques confirmed the stability, morphology, and polymer compatibility of the formulations. In vivo studies revealed significantly higher drug concentrations in brain tissue for the optimized formulations (LCH3 and LC3) compared to pure lorazepam, highlighting their potential to bypass the blood-brain barrier and achieve rapid therapeutic effects. These findings demonstrate the promise of nasal microspheres as an innovative, efficient, and patient-friendly approach for the targeted treatment of central nervous system disorders like epilepsy. Future work should focus on the development of suitable delivery devices for clinical application.

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