International Journal of Research in Pharmacy and Pharmaceutical Sciences
www.pharmacyjournal.in
ISSN: 2455-698X
Received: 10-06-2026, Accepted: 10-07-2026, Published: 25-07-2026
Volume 11, Issue 3, 2026, Page No. 8-12

Formulation and evaluation of nasal pH responsive in situ gel of Levetiracetam loaded invasomes

Rohit Singh*, Urvesh Singh Narwaria
IPS College of Pharmacy, Gwalior, Madhya Pradesh, India
Corresponding Author: Rohit Singh
DOI: https://doi.org/10.66856/ijrpps.2026.11.3.11071

Abstract
Levetiracetam, a widely used antiepileptic drug, suffers from limitations such as extensive first-pass metabolism and variable oral bioavailability. To address these challenges, invasome-based intranasal mucoadhesive in situ gels were developed and evaluated. Invasomes were prepared using soy lecithin, ethanol, and limonene via thin layer hydration, and characterized for particle size, zeta potential, and entrapment efficiency. The optimized formulation (F3) exhibited the smallest particle size (143.7 nm), highest entrapment efficiency (84.6%), and sustained drug release over 12 hours. Incorporation into thermoresponsive gels using Pluronic F127, Pluronic F68, and chitosan resulted in formulations with suitable gelation temperature (31–33°C), viscosity, and mucoadhesive properties compatible with nasal pH. *In vitro* release studies demonstrated that the invasome-loaded gel (LNG4) provided prolonged drug release up to 8 hours (70.4%), compared to rapid release from levetiracetam solution. These findings suggest that invasome-based intranasal gels can enhance bioavailability, sustain drug release, and improve brain targeting of levetiracetam, offering a promising alternative for epilepsy management.

Keywords: Levetiracetam, epilepsy, invasomes, intranasal drug delivery, mucoadhesive in situ gel

Introduction

Epilepsy is a chronic neurological disorder characterized by recurrent seizures, often requiring long-term pharmacological management [1]. Levetiracetam, a widely prescribed antiepileptic drug, demonstrates favorable efficacy and safety but suffers from limitations such as extensive first-pass metabolism and variable oral bioavailability [2]. These challenges necessitate the exploration of alternative drug delivery strategies that can enhance therapeutic outcomes while minimizing systemic side effects.

Intranasal drug delivery has emerged as a promising route for central nervous system (CNS) targeting due to its ability to bypass hepatic metabolism and provide rapid absorption through the highly vascularized nasal mucosa [3, 5]. Moreover, the incorporation of particulate carrier systems can further improve drug stability, prolong residence time, and enable controlled release. Among these, invasomes, novel vesicular carriers composed of phospholipids, ethanol, and terpenes, have shown remarkable potential in enhancing drug penetration and sustaining release profiles [6, 8]. Their nanoscale size and flexible bilayer structure facilitate improved mucosal adhesion and efficient transport across biological membranes.

Given these advantages, the present study was designed to develop and evaluate invasome-based intranasal mucoadhesive in situ gels of levetiracetam. The approach aims to overcome the limitations of conventional oral therapy by improving bioavailability, sustaining drug release, and enhancing brain targeting. By combining invasomes with thermoresponsive and mucoadhesive polymers such as Pluronic F127, Pluronic F68, and chitosan, the formulation is expected to provide optimal gelation at nasal temperature, prolonged retention, and controlled drug delivery. This work thus represents a step toward innovative intranasal therapies for epilepsy management.

Material and Methods

Levetiracetam has been purchased from Yarrow Pharmaceuticals, Mumbai. Soy Lecithin has been purchased from Himedia, chitosan was purchased from Oxford Chemicals. Pluronic F68 and Pluronic F127 were purchased from Yarrow Pharmaceuticals. Melting point apparatus (Biotechnics India, BTI-34), digital pH meter (Labtronics, LT-53), UV/Vis double beam spectrophotometer (Labtronics, LT-2201), Viscometer (Brookfield, DV-1) were used for the study.

Preformulation Studies

Preformulation parameters like organoleptic characters, melting point range, and qualitative solubility were studied using reported procedures [7]. Calibration curve of levetiracetam was prepared in distilled water by UV-Visible spectrophotometer. Briefly, an accurately weighed quantity of 10 mg levtiracetam pure drug was taken in a 10 mL volumetric flask. Sufficient quantity of distilled water was added to it and shaken well until the drug completely dissolved. From this, 1 mL of the solution was pipetted out and made up to 100 mL with distilled water. From this 0.5, 1.0, 1.5, 2.0 and 2.5 mL of solutions are pipetted out in separate standard flasks and the volume was made up to 10 mL with distilled water. The absorbance is measured at 220 nm uisng UV-Spectrophotometer [9].

Preparation of Invasomes

Thin layer hydration technique was used for preparing the invasomes. Limonene was be used as terpenes, soy lecithin would be used as the lipid to prepare vesicles. Different levetriracetam-loaded invasomes were prepared using the conventional thin layer hydration technique. Accurately weighted amount of soy lecithin (200 mg) was dissolved in 10-mL ethanol and to it various concentration of terpene (limonene) was added and stirred until a clear solution was obtained. Levetiracetam (1% w/v) was dissolved in a 1%

v/v hydro-ethanolic solution. Both the solutions were separately sonicated at 60°C for 30 min to assure homogeneity. The organic solvent (ethanol) from the oil phase was evaporated by rotatory evaporator at 120 rpm at 60°C for 15 min to obtain a clear film on the walls of the flask. The deposited lipid film was then hydrated with aqueous phase by rotation at 120 rpm for 1 h at 60°C, followed by hand shaking for 5 min. The mixture was finally sonicated at 60°C for 30 min to obtain the invasome formulation [10].

Table 1: Formulation formula for invasomes

S.No.	Ingredient	F1	F2	F3
1	Levetiracetam (%)	1.0	1.0	1.0
2	Soy Lecithin (mg)	200	200	200
3	Methanol-chloroform (2:1 v/v) (mL)	10	10	10
4	Limonene (% w/v)	0.5	1.0	1.5

Evaluation of Invasomes [11]

Entrapment Efficiency

To determine the amount of drug entrapped in the invasomes, 1-mL of levetiracetam-loaded invasome dispersion was centrifuged at 13,000 rpm at for 60 min. The clear supernatant was siphoned off carefully to separate the unentrapped levetiracetam and the supernatant was analyzed by UV spectrophotometer. Sediment was treated with 1 ml of 0.1% Triton X 100 to lyse the vesicles and then diluted to 100 ml with water and levetiracetam was analyzed by UV spectrophotometry. Amount of levetiracetam in supernatant and sediment gave a total amount of levetiracetam in 1 ml dispersion. The percent entrapment was calculated using the formula:

% entrapment= amount of levetiracetam in sediment/amount of levetiracetam added ×100

Particle size

The particle size of the prepared invasomes was measured using the dynamic light-scattering by Malvern Zetasizer at temperature of 25 ± 2°C. The zeta potential of a selected levetiracetam -loaded invasome was also performed using 90° scattering angle at temperature of 25 ± 2°C.

***In vitro* release**

The *in vitro* release of levetiracetam from different levetiracetam -loaded invasomes was evaluated using the dialysis bag diffusion technique [12]. Amount of levetiracetam -loaded invasomes (equivalent to 1 mg levetiracetam) was separated by centrifugation at 13,000 rpm for 1 h, then re-dispersed in 1-mL 3% (v/v) ethanolic aqueous solution and placed in a cellulose membrane bag. The dialysis bag was tightly closed at both ends and immersed in a stoppered bottle containing 100-mL phosphate buffer saline (PBS; pH = 7.4) representing the receptor compartment. The bottle was placed in a water bath shaker, stirred at 100 rpm, and maintained at 37 ± 0.5°C. At pre-determined time intervals, 3-mL sample of the receptor compartment was withdrawn and replaced with an equal volume of fresh medium to keep constant volume. The samples were properly diluted and analyzed for the amount of drug released was determined by UV spectrophotometry.

Formulation of invasomal in situ gel

Determination of gelation temperature

Temperature at which the liquid (sol) phase converts to gel form is termed as gelation temperature. The sol–gel transition temperature of the prepared in-situ gel formulations was determined by visual inspection method [13]. Briefly the solutions of Pluronic F127 in the concentrations (18–23 % w/v) were prepared by stirring on a magnetic stirrer in a transparent 10 ml glass bottle sealed with paraffin. The vial was heated at constant rate with an increment of 1°C and the temperature at which the magnetic bead stopped moving due to gelation was considered as gelation temperature. Gels which showed gelation temperature very close to nasal temperature (32-34°C) were selected for further evaluation. Effect of chitosan and Pluronic F68 on phase transition temperature was evaluated by dispersing different concentration (0.1–0.5 % w/v) in optimized Pluronic F127 solutions.

In situ gel formulation

Poloxamer F127 and Pluronic F68 gel was prepared by dissolving the optimized concentration in cold (4°C) water under constant stirring. The hazy solution formed was kept in refrigerator (2–4°C) overnight for complete dissolution resulting in a clear solution. Chitosan dissolved in 2% acetic acid was added slowly to the pluronic solution with continuous stirring at 4°C (Table 2) [14]. The invasomes were dissolved in ethanol and added to this mixture under continuous stirring to obtain the microemulsion. The pH of the solution was adjusted by addition of 0.1N NaOH solution to obtain gel formulation.

Table 2: Composition of intranasal gel formulations

Formulation Code	Invasome (% w/v)	Puronic F127 (%w/v)	Pluronic F68 (%w/v)	Chitosan (%w/v)
LNS	Pure drug solution (0.5%)	--	--	--
LNG1	0.5	18	--	0.1
LNG2	0.5	18	--	0.2
LNG3	0.5	18	--	0.3
LNG4	0.5	18	0.60	0.1
LNG5	0.5	18	0.60	0.2
LNG6	0.5	18	0.60	0.3

Evaluation of in situ gel

pH

The pH of each formulation was determined by pH meter. Initially, the pH meter was calibrated using standard buffer solutions of pH 4 and pH. 1 mL of the formulation was diluted with distilled water and the pH of the solution was recorded by dipping the electrode in the solution.

Viscosity and rheological studies

Viscosity of in situ gel system was determined using Brook field viscometer DV-1. Temperature of 37±0.5ºC was maintained and the spindle was lowered perpendicularly into both in situ sol and gel formulations which were placed in a beaker. The viscosity of each formulation was determined by applying 100 rpm speed.

Drug content

The drug content of the prepared gel was carried out by dissolving accurately weighed quantity of gel and calculating the amount of drug present in the solution by measuring the absorbance of solution using UV spectrophotometer.

Rheological Studies

The measurement of viscosity of prepared in situ gel was done with Brookfield viscometer. The in situ formulations were rotated for 2 minutes at different speeds (10-100 rpm) for selected spindle. At each speed the corresponding dial reading was noted. The viscosity of different in situ gel formulations was measured at different speeds at room temperature.

Gel Strength

Gel strength was determined by placing a standard weight of 35 g onto 50 g of thermoreversible gel (placed in 100 ml beaker) maintained at gelation temperature using controlled water bath. The time in seconds by the weight to penetrate 5 cm deep into the container was recorded as gel strength.

***In vitro* drug release**

Drug release from gel was determined by using Franz diffusion cell. Artificial dialysis membranes were soaked in receptor medium for 2h prior to use. Phosphate buffer saline (12 ml) pH 6.4 was added into the receptor chamber maintained at 34 ± 1°C. Gel equivalent to 2.5 mg of drug was placed into donor compartment and the setup was kept on stirring. Aliquots of 1ml were withdrawn at predetermined time intervals from receptor compartment and replaced with fresh buffer till 12 h. The samples were diluted suitably and analyzed spectrophotometrically at 220 nm and the amount of drug released was determined using calibration curve.

Results and Discussion

The physical characterization of the procured levetiracetam sample was carried out in order to confirm the identity of the drug. The drug was white crystalline powder with melting range of 120-121°C and was soluble in water, methanol and ethanol. The λmax of levetriracetam was determined by scanning a 10µg/mL solution of the drug using UV spectrophotometer from 200-400 nm. The λmax was found to be 220 nm and was hereafter used for determination of the drug in solution. The absorbance of 5 to 25 µg/mL solutions was measured at 220 nm by UV spectrophotometer and calibration curve was constructed (Figure 1).

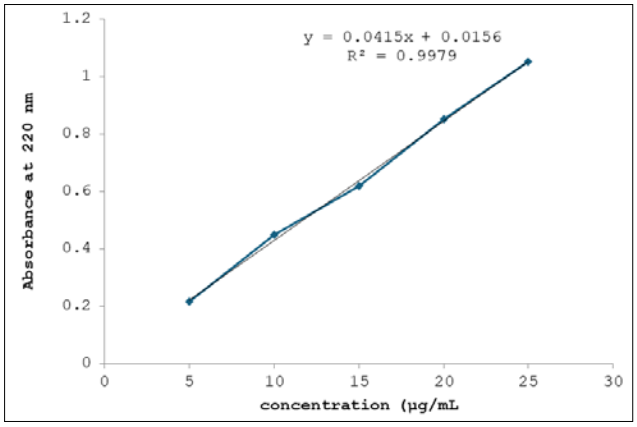

Fig 1: Calibration curve of levetiracetam

Particle size and zeta potential of invasomes

The particle size of the invasomes was determined by Malvern zeta sizer and the average particle size of the formulations ranged from 143.7 to 244.5 nm (Table 3). The size was found to decrease with the increasing concentration of the terpene. The zeta potential of the invasomal formulations -19.4 mV to -24.7 mV. A similar effect of increasing the concentration of terpene was observed in a study of invasomes of adapalene by Targhotra and Gupta [15].

Entrapment efficiency of invasomes

The entrapment efficiency was determined by ultrafiltration method using centrifugal tubes. The entrapment of the doxycycline in the invasomes was found to enhance with the increased concentration of the terpene. The drug entrapment was found to be between 65.84 to 84.60 % (Table 3). The higher entrapment could be attributed to the lipophilicity of limonene which enhances the tendency to form lipoidal interactions with the drug [16].

Table 3: Particle size, zeta potential and entrapment efficiency of invasomes

Formulation	Particle size	Zeta Potential	% EE
F1	244.5 nm	-19.4 mV	65.84
F2	213.8 nm	-21.5 mV	78.26
F3	143.7 nm	-24.7 mV	84.60

***In vitro* drug release**

The release of levetiracetam from invasomes was studied for a period of 12 hours and the invasomes were able to sustain the release of levetiracetam for duration of 12 hours (Figure 2).

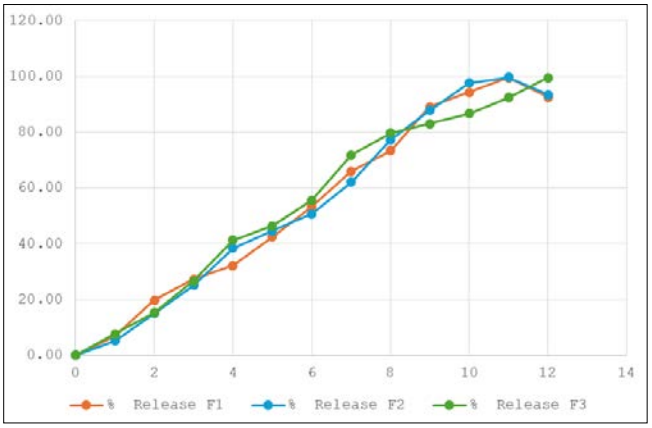

Fig 2: *In vitro* release of levetiracetam from invasomes

F3 with lowest particle size and highest release of levetiracetam was selected as the best formulation of in situ intranasal gel.

Determination of gelation temperature

Phase transition temperature determination is a preliminary step in the formulation of the in situ gel. Gelation temperature of gel formulations is shown in Table 6.5 which suggests that Pluronic F127 in the concentration of 18% w/v showed best results for phase transition at 31-33°C. As the concentration of Pluronic increased -from 18 to 23 %, transition temperature decreased from 34 to 25 °C.

The addition of Pluronic F68 also affected the gelation behavior of the formulations. The effect of varying concentration of Pluronic F68 on gelation temperature revealed that the gelation temperature was inversely related

to the concentration fo Pluronic F68. Increasing the concentration of Pluronic F68 led to a decrease in gelation temperature of the formulations (Table 4). A concentration of 0.75 and higher of Pluronic F68 decreased the gelation temperature to less than 25°C making the concentrations unsuitable for in situ intranasal gel delivery.

Table 4: Effect of Pluronic F68 on gelation temperature

S.No.	Pluronic F127 (%w/v)	Pluronic F68 (%w/v)	Gelation Temperature (°C)
1	18	0.6	32-35
2	18	0.75	25-27
3	18	0.9	23-24
4	18	1.05	20-22

The in situ intranasal gel formulations were prepared using either Pluronic F127 (18%w/v) with chitosan or mixture of Pluronic F127 (18%w/v) and F68 (0.6%w/v) with chitosan. Chitosan was used as a pH sensitive mucoadhesive agent for intranasal delivery. At pH below 6.2, chitosan usually remains as a solution whereas above pH 6.2 it forms gel. The pH of nasal cavity is 6.8 and hence chitosan would be helpful in mucoadhesion, leading to prolonged residence of the gel in nasal cavity and thereby help in higher absorption of the drug from the invasomes. The effect of chitosan concentration on the viscosity of formulations is shown in Figure 3.

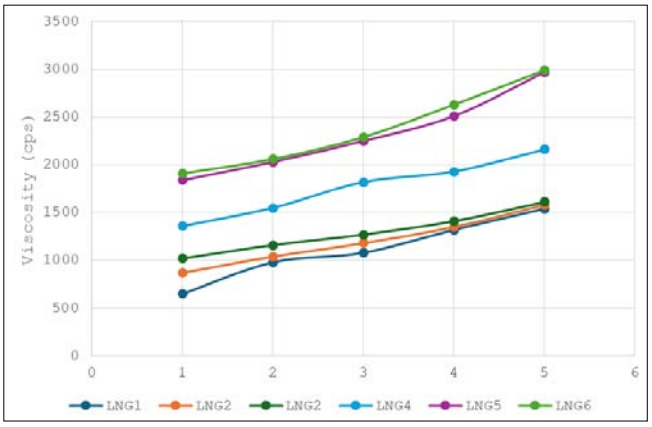

Fig 3: Effect of chitosan on in situ gelling

Evaluation of the in situ gel formulations

Physicochemical properties

The pH of all the microemulsion was 5.8 - 6.3 and the gel form of the same was found to be from 6.7 to 6.8 which is in between nasal pH (5.5 to 6.8). This ascertains that all the formulations are compatible with nasal mucosa. The formulations LNG1, LNG2, LNG4 and LNG5 were found to be clear while LNG3 and LNG6 were turbidity appearance revealing that clarity of the gel is decreased on increasing the concentration of chitosan in the formulations. The results of pH, clarity, drug content, viscosity, gelling time and gel strength are presented in Table 5.

Table 5: Physicochemical properties of the in situ gel formulations

Formulation code	pH	Clarity	Drug content (%)	Viscosity (cps)		Gel Strength (g)	Gelling time (sec)
				Sol (at 25°C)	Gel (at 31°C)		
LNG1	6.71	++	92.7	650	1320	4.7	10
LNG2	6.73	++	95.3	870	1350	5.4	9
LNG3	6.82	+	95.1	1020	1410	6.2	7
LNG4	6.73	++	95.9	1360	1930	6.9	9
LNG5	6.76	++	96.8	1840	2510	7.6	7
LNG6	6.80	+	97.4	1910	2630	7.9	5

The most important feature for intranasal in situ gel is viscosity of the formulation. A formulation suitable for application to the nasal cavity should ideally have a low viscosity when applied and after administration should have a high viscosity in order to stay at the application site. All the formulations exhibited a chitosan concentration dependent increase in viscosity. Viscosity of the both in situ sol and in situ gel was examined at 100 rpm. LNG6 formulation was having maximum viscosity. The viscosity of LNG4 (1360 in sol to 1930 in gel) was taken as optimum. Viscosity of the sol formulation ranged between 650 to 1910 cps while that of the in situ gel ranged between 1320 to 2630 cps.

Rheological Studies

Rheological behaviour study is an important parameter for the in situ gel. The viscosity of formulations should be in an optimum range which improves its ease of administration. The flow curve (viscosity against speed / rpm) of the formulation (LNG4) indicated that for the all the polymer concentrations, the formulations exhibited the properties of pseudoplastic systems with shear thinning. The prepared formulations tend to thin when being exposed to shearing force and therefore tend to be easily syringeable and spreadable. The effect of agitation speed on viscosity is presented in Figure 4.

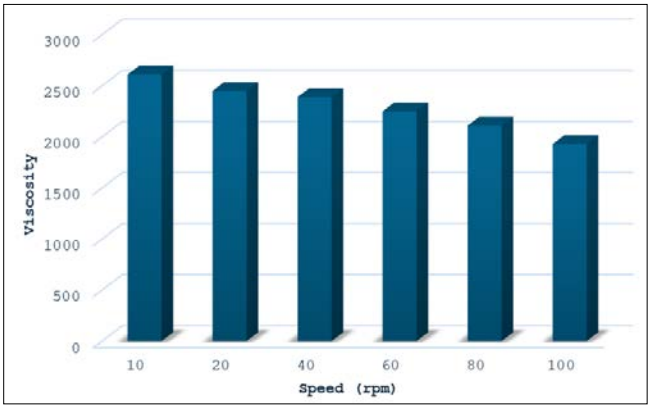

Fig 4: Rheological behavior of LNG4

***In vitro* drug release from in situ gel**

The *in vitro* release studies of drug loaded in situ gel was carried out for 20 min in PBS pH 6.8. PBS of pH 6.8 was selected as medium for drug absorbance since it resembles nasal pH. Throughout the study the pH and temperature were kept constant. The release study was compared with levetiracetam solution and it was found that the levetiracetam solution released 100.2% drug in 12 min whereas only 1.4% drug was released from the in-situ gel in the same duration (Figure 5).

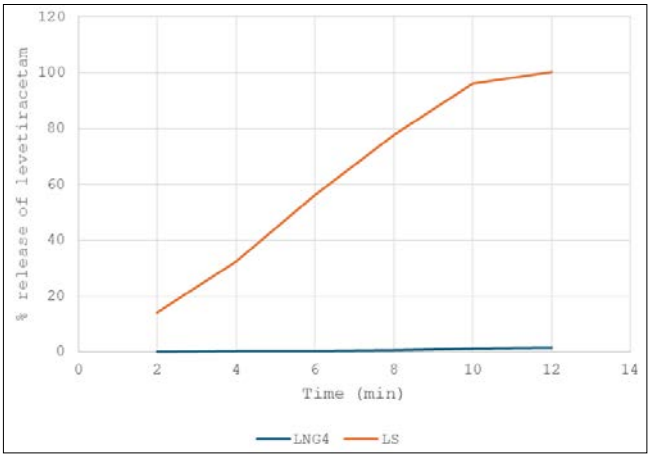

Fig 5: Comparative drug release profile from LNG4 and LS

On the other hand, on continuing the release study for 8 hours it was found that the release of levetiracetam from the levetiracetam-invasome loaded in situ gel could be prolonged for more than 8 hours (70.4%) suggesting that loading of the invasomal formulation was effective and could help in enhanced delivery of drug to the brain via the nasal route (Figure 6). .

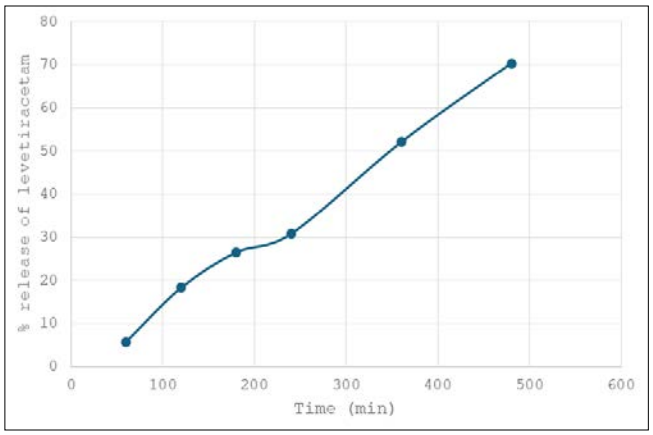

Fig 6: Release of levetiracetam from LNG4

Conclusion

The present study successfully developed and characterized invasome-based intranasal mucoadhesive in situ gels of levetiracetam. The optimized invasome formulation demonstrated nanoscale particle size, high entrapment efficiency, and sustained drug release. Incorporation into thermoresponsive gels provided suitable gelation temperature, viscosity, and mucoadhesive properties, ensuring compatibility with nasal physiology. Comparative release studies confirmed that invasome-loaded gels significantly prolonged drug release compared to pure drug solution, highlighting their potential to bypass first-pass metabolism and enhance brain delivery. Overall, this approach represents a promising strategy for improving the therapeutic efficacy of levetiracetam in epilepsy management and may serve as a foundation for future clinical translation of intranasal invasomal drug delivery systems.

References

1. Malik F, Nikhil V, Venkata Aravind Kumar P, Vasadi L, Manasa NR. A literature review on epilepsy. International Journal of Creative Research Thoughts,2024:12(6):76-763.
2. Tripathi KD. Essentials of Medical Pharmacology, 6th Edition. Jaypee Brothers Medical Publishers (p) Ltd.; New Delhi, 2010, 406.
3. Jansson B, Hägerström H, Fransén N, Edsman K, Björk E. The influence of gellan gum on the transfer of fluorescein dextran across rat nasal epithelium *in vivo*. EurJPharmaceutBiopharmaceut,2005:59(3):557-564.
4. Mahajan HS, Gattani SG. Gellan gum based microparticles of metoclopromide hydrochloride for intranasal delivery: development and evaluation. Chem Pharm Bull,2009:57(4):388-392.
5. Cao SL, Ren XW, Zhang QZ, Chen E, Xu F, Chen J *et al*. In situ gel based on gellan gum as new carrier for nasal administration of mometasone furoate. Int J Pharmaceut,2009:365(1):109-115.
6. Samir B, El-Kamel A, Zahran N, Heikal L. Resveratrol-loaded invasome gel: A promising nanoformulation for treatment of skin cancer. Drug Delivery and Translational Research, 2024. https://doi.org/10.1007/s13346-024-01534-9
7. Salih OS, Al-Akkam EJ. Preparation, *In vitro*, and Ex *vivo* Evaluation of Ondansetron Loaded Invasomes for Transdermal Delivery. Iraqi Journal of Pharmaceutical Sciences, 2023, 32(3). DOI: https://doi.org/10.31351/vol32iss3pp71-84
8. Verma H, Pal P, Joshi D. Formulation, Development and Evaluation of Invasomes Loaded Gel for Fungal Treatment. Sch Acad J Pharm,2022:11(7):105-108.
9. Gawale K, Syed SM. Development and Validation of UV Spectroscopic and RP-HPLC Method for Determination of Levetiracetam in Bulk and Combined Dosage Form. Journal of Pharmaceutical and Applied Chemistry,2021:7(1):25-32.
10. Shah SM, Ashtikar M, Jain AS, Makhija DT, Nikam Y, Gude RP *et al*. LeciPlex, invasomes, and liposomes: a skin penetration study. International Journal of Pharmacy,2015:490(1–2):391–403.
11. Ruckmani K, Jayakar B, Ghosal SK. Nonionic surfactant vesicles (niosomes) of cytarabine hydrochloride for effective treatment of leukemias: encapsulation, storage, and *in vitro* release. Drug Development and Industrial Pharmacy,2000:26(2):217–222.
12. Yang SC, Lu LF, Cai Y, Zhu JB, Liang BW, Yang CZ. Body distribution in mice of intravenously injected camptothecin solid lipid nanoparticles and targeting effect on brain. Journal of Controlled Release,1999:59(3):299–307.
13. Kempwade K, Taranalli A. Formulation and evaluation of thermoreversible, mucoadhesive in situ intranasal gel of rizatriptan benzoate. Journal of Sol-Gel Science and Technology,2014:72:43-48.
14. Schmolka IR. Artificial skin I. Preparation and properties of pluronic F-127 gels for treatment of burns. Journal of Biomedical Materials Research,1972:6:571-582.
15. Targhotra M, Gupta M. Development and characterization of effective topical formulation for adapalene loaded invasomes for acne management. Indian J Pham Sci Res,2020:10(1):1-8.
16. Pauli A. Relationship between lipophilicity and toxicity of essential oils. International Journal of Essential Oil Therapeutics,2008:2:60-68.