



Original Research Paper

Stimuli-Responsive Carbamazepine-Loaded Chitosan Nanogel Systems for Targeted Nose-to-Brain Transport in Drug-Resistant Temporal Lobe Epilepsy: Mechanistic Insights and Ex Vivo Evaluation

Pankaj Mohan Pimpalshende¹, Saravanan K.S², Jyothirmayee Devineni^{3*}, B. Sree Giri Prasad⁴, Pratibha Gautam⁵, B. R. Asokan⁶, Lalitha Gedela⁷, Bhavik Sharma⁸,

¹Department of Pharmaceutics, Hi-Tech College of Pharmacy Chandrapur 442406.

^{2,6}Department of Pharmacology, Trichy SRM Medical College Hospital & Research Centre, SRM Nagar, Irungalur, Trichy – 621105 India.

^{3,7}Department of Pharmaceutics. University College of Pharmaceutical Sciences, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur. Pin:522510,

⁴Department of Pharmaceutics, Nalla Narasimha Reddy Education Society's Group of Institutions, Medchal Malkajgiri, Ghatkesar, Hyderabad, Telangana, India Pin- 500088.

⁵Department of Pharmacognosy, Shri Ramswaroop Memorial University, Tindola, Deva Road, Lucknow, Uttar Pradesh Pin code- 225003.

⁸Department of Pharmacy, Madhav University, Pindwara, Sirohi, Rajasthan, 307026.

Corresponding Author**

Jyothirmayee Devineni

Department of Pharmaceutics. University College of Pharmaceutical Sciences, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur. Pin:522510.

Email id: - djyothirmayee9@gmail.com

Received: 13th July, 2026; Revised: 27th July, 2026; Accepted: 27th August, 2026; Available Online: 29th August, 2026

Abstract

Drug-resistant temporal lobe epilepsy (TLE) remains a major clinical challenge due to limited drug penetration across the blood–brain barrier (BBB) and systemic side effects associated with conventional therapy. The present study focuses on the development of stimuli-responsive carbamazepine (CBZ)-loaded chitosan nanogels for targeted nose-to-brain delivery as an alternative therapeutic strategy. Chitosan-based nanogels were prepared using ionic gelation and optimized to achieve nanoscale particle size, high encapsulation efficiency, and favorable surface charge for enhanced mucoadhesion and permeation. Physicochemical characterization confirmed uniform morphology, stability, and stimuli-responsive gelation behavior under physiological conditions. In vitro drug release studies demonstrated a sustained and controlled release profile following non-Fickian diffusion kinetics, ensuring prolonged therapeutic action. Ex vivo permeation studies using nasal mucosa revealed significantly enhanced drug transport compared to pure CBZ, attributed to chitosan-mediated opening of tight junctions and increased residence time. Mucoadhesion studies further supported prolonged nasal retention, reducing mucociliary clearance and improving absorption efficiency. Histopathological evaluation confirmed the safety and biocompatibility of the formulation, showing no significant tissue damage or irritation. The findings suggest that CBZ-loaded chitosan nanogels effectively enhance drug delivery to the brain via the intranasal route, overcoming limitations of conventional formulations. This approach provides improved bioavailability, sustained release, and targeted delivery, making it a promising strategy for managing drug-resistant epilepsy. Further in vivo and clinical investigations are warranted to establish its therapeutic potential and translational applicability.

Keywords: Carbamazepine, Chitosan Nanogels, Nose-to-Brain Delivery, Temporal Lobe Epilepsy, Mucoadhesion, Controlled Release

1. Introduction

Epilepsy is a chronic neurological disorder characterized by recurrent, unprovoked seizures resulting from abnormal and excessive neuronal

*Corresponding Author:

Received: 13th July, 2026; Revised: 27th July, 2026; Accepted: 27th August, 2026; Available Online: 29th August, 2026

(DOI): 10.70102/AEJ.2026.18.4s.28

activity in the brain. It affects over 50 million people worldwide, making it one of the most prevalent neurological conditions with significant socioeconomic and healthcare burdens. Among the various forms of epilepsy, temporal lobe epilepsy (TLE) is the most common and is frequently associated with drug resistance, where patients fail to respond adequately to conventional antiepileptic drugs (AEDs) (Beghi, 2020). The pathophysiology of TLE involves complex neuronal network alterations, hippocampal sclerosis, neuroinflammation, and excitotoxicity, which collectively contribute to recurrent seizure activity and disease progression. Despite the availability of multiple AEDs, approximately one-third of patients remain refractory to treatment, necessitating the exploration of innovative therapeutic strategies (Shorvon, 2011). Carbamazepine (CBZ) is a first-line antiepileptic drug widely prescribed for focal seizures and TLE due to its proven efficacy in stabilizing neuronal membranes and reducing seizure frequency. However, its clinical utility is limited by several pharmacokinetic and pharmacodynamic challenges, including poor aqueous solubility, variable oral bioavailability, hepatic metabolism, and systemic side effects such as dizziness, drowsiness, and hepatotoxicity. Moreover, the presence of the blood–brain barrier (BBB) significantly restricts the efficient delivery of CBZ to the central nervous system (CNS), often requiring higher systemic doses that increase the risk of adverse effects. These limitations are particularly problematic in drug-resistant epilepsy, where achieving therapeutic concentrations in the brain becomes increasingly difficult (Kasri et al., 2024; Sá Filho et al., 2024).

In recent years, the field of nanotechnology has introduced promising approaches to overcome these limitations, particularly through the development of nanocarrier-based drug delivery systems. Nanocarriers such as nanoparticles, liposomes, nanoemulsions, and nanogels have demonstrated the ability to enhance drug solubility, improve stability, and facilitate targeted delivery to the brain (Chen et al., 2022). Among these, nanogels, which are three-dimensional, hydrophilic polymer networks capable of swelling in aqueous environments, have gained significant attention due to their unique physicochemical properties, including high drug loading capacity, controlled release behavior, and biocompatibility. These systems can be engineered to respond to specific physiological stimuli such as pH, temperature, or ionic strength, enabling stimuli-responsive drug release at the target site (Mu et al., 2020).

One of the most promising strategies for delivering drugs to the brain is the nose-to-brain route, which bypasses the BBB and allows direct access to the CNS

via the olfactory and trigeminal neural pathways. Intranasal administration offers several advantages, including rapid onset of action, non-invasive delivery, avoidance of first-pass metabolism, and improved patient compliance. However, challenges such as mucociliary clearance, enzymatic degradation, and limited permeability of the nasal mucosa must be addressed to achieve effective drug delivery. To overcome these barriers, researchers have focused on incorporating mucoadhesive and permeation-enhancing polymers into nanocarrier systems (Keller et al., 2022). Chitosan, a naturally derived polysaccharide obtained from chitin, has emerged as a highly suitable polymer for nasal drug delivery applications. Its biocompatibility, biodegradability, and mucoadhesive properties make it an ideal candidate for enhancing drug retention in the nasal cavity. Importantly, chitosan has the ability to transiently open tight junctions between epithelial cells, thereby facilitating paracellular transport of drugs across the nasal mucosa. This property significantly enhances drug permeation and absorption into the systemic circulation and brain. Furthermore, chitosan-based nanogels can be functionalized or crosslinked to create stimuli-responsive systems, allowing precise control over drug release kinetics and improving therapeutic outcomes (Omidian et al., 2024).

The integration of carbamazepine into chitosan-based nanogel systems represents a novel and promising approach for the treatment of drug-resistant TLE. By encapsulating CBZ within nanogels, it is possible to improve its solubility, protect it from degradation, and achieve sustained release profiles. Additionally, the nanoscale size of these carriers facilitates efficient transport through the nasal mucosa and enhances accumulation in the brain. Studies have demonstrated that intranasal delivery of CBZ-loaded nanocarriers results in higher brain concentrations, improved anticonvulsant efficacy, and reduced systemic toxicity compared to conventional formulations (Liu et al., 2018).

Another critical aspect of advanced nanogel systems is their stimuli-responsive behavior, which allows them to respond to specific physiological conditions such as temperature or pH changes. For instance, thermoresponsive nanogels can undergo sol–gel transitions at nasal cavity temperatures, forming a gel-like structure that enhances residence time and drug retention. Similarly, pH-responsive systems can release the drug in response to local environmental changes, ensuring targeted and controlled delivery. These features are particularly advantageous for managing chronic conditions like epilepsy, where maintaining consistent therapeutic drug levels is essential (Delgado-Pujol et al., 2025). *Ex vivo* and *in*

vitro studies play a crucial role in evaluating the performance of these nanogel systems before clinical application. Ex vivo permeation studies using excised nasal mucosa (e.g., goat or sheep) provide valuable insights into drug transport mechanisms, permeability coefficients, and tissue compatibility. Such studies have consistently shown that chitosan-based nanogels enhance drug permeation without causing significant tissue damage, highlighting their safety and efficacy. Additionally, histopathological analyses confirm the non-toxic nature of these systems, supporting their potential for long-term use (Boyuklieva et al., 2023). Despite these promising advancements, several challenges remain in translating nanogel-based drug delivery systems from the laboratory to clinical practice. Issues such as large-scale manufacturing, formulation stability, reproducibility, and regulatory approval must be addressed. Furthermore, comprehensive in vivo studies and clinical trials are necessary to validate the efficacy and safety of these systems in human populations. Nevertheless, the growing body of evidence supporting the use of nanotechnology in epilepsy treatment underscores its potential to revolutionize current therapeutic approaches (Parvin et al., 2025).

In this context, the present study focuses on the development and evaluation of stimuli-responsive carbamazepine-loaded chitosan nanogel systems for targeted nose-to-brain delivery in drug-resistant temporal lobe epilepsy. By combining the advantages of nanotechnology, mucoadhesion, and stimuli responsiveness, this approach aims to overcome the limitations of conventional CBZ therapy and provide a more effective and safer treatment option. The study further investigates the mechanistic aspects of drug delivery and evaluates the performance of the developed system through ex vivo permeation studies, offering valuable insights into its potential clinical applicability (Abou-Taleb & El-Ganainy, 2023).

2. Mechanism of Carbamazepine in Epilepsy

Carbamazepine (CBZ) is a widely used first-line antiepileptic drug, particularly effective in focal seizures and temporal lobe epilepsy (TLE). Its therapeutic efficacy arises from a multifaceted mechanism of action that primarily targets neuronal excitability, synaptic transmission, and long-term epileptogenic processes. Although its principal activity involves modulation of voltage-gated ion channels, accumulating evidence indicates that CBZ also influences neurotransmitter systems and neuroprotective pathways, contributing to both acute seizure suppression and long-term disease modification (Tolou-Ghamari et al., 2013).

2.1 Voltage-Gated Sodium Channel Blockade

The primary mechanism of carbamazepine involves the inhibition of voltage-gated sodium (Nav) channels,

which are critical for the initiation and propagation of action potentials in neurons. CBZ binds preferentially to the inactive state of sodium channels, prolonging their inactivation and thereby reducing the ability of neurons to fire repetitively at high frequencies. This use-dependent blockade is particularly important in epileptic conditions, where neurons exhibit excessive and sustained depolarization (Jo & Bean, 2014). By stabilizing hyperexcited neuronal membranes, CBZ effectively suppresses the paroxysmal depolarization shifts that underlie seizure activity. This results in decreased propagation of abnormal electrical discharges across neuronal networks, especially in limbic structures such as the hippocampus, which are heavily implicated in TLE. Furthermore, CBZ's action is selective for rapidly firing neurons, meaning it preferentially targets pathological activity while sparing normal neuronal function. This selectivity contributes to its clinical efficacy and tolerability (Rocchi et al., 2025).

2.2 Modulation of Synaptic Transmission

In addition to its effects on sodium channels, carbamazepine modulates synaptic neurotransmission, particularly by influencing the balance between excitatory and inhibitory signaling in the brain. One of its key actions is the reduction of glutamate release, the principal excitatory neurotransmitter involved in seizure generation and propagation. Experimental studies have demonstrated that CBZ inhibits sodium channel-mediated glutamate release from presynaptic terminals, thereby dampening excitatory synaptic transmission (Wu et al., 2019). CBZ may also indirectly enhance inhibitory neurotransmission, although its effects on gamma-aminobutyric acid (GABA) systems are less pronounced compared to other antiepileptic drugs. Additionally, evidence suggests that CBZ interacts with adenosine signaling pathways, particularly through modulation of adenosine receptors, which play a crucial role in suppressing neuronal excitability and seizure activity. Adenosine acts as an endogenous anticonvulsant, and CBZ's ability to influence this system may further contribute to its therapeutic effects (Okada et al., 2019). Moreover, CBZ has been shown to affect voltage-gated calcium channels, which are involved in neurotransmitter release and synaptic plasticity. By modulating calcium influx, CBZ can further regulate synaptic activity and reduce excitatory transmission. Collectively, these effects shift the excitatory-inhibitory balance toward a more stable neuronal state, thereby preventing seizure initiation and spread (Ho et al., 2020).

2.3 Neuroprotective and Anti-Kindling Effects

Beyond its immediate anticonvulsant properties, carbamazepine exhibits neuroprotective and anti-kindling effects, which are particularly relevant in

chronic and drug-resistant epilepsy. The phenomenon of kindling refers to the progressive increase in seizure susceptibility following repeated subthreshold stimulation, a process believed to contribute to epileptogenesis. CBZ has been shown to inhibit kindling development, suggesting a role in modifying disease progression (Tiwari et al., 2026). One proposed mechanism underlying these effects is the ability of CBZ to reduce neuroinflammation and oxidative stress, both of which are implicated in neuronal damage and epileptic remodeling. CBZ has been reported to attenuate the release of pro-inflammatory cytokines and decrease astroglial glutamate release, thereby protecting neurons from excitotoxic injury. Additionally, its interaction with adenosine pathways may further enhance neuroprotection, as adenosine is known to exert anti-inflammatory and neuromodulatory effects (Ma et al., 2022). These long-term actions are especially important in TLE, where structural and functional changes in the hippocampus contribute to persistent seizure activity. By limiting neuronal hyperexcitability, synaptic reorganization, and inflammatory responses, CBZ not only controls seizures but may also slow the progression of epilepsy (Methods in Medicine, 2023).

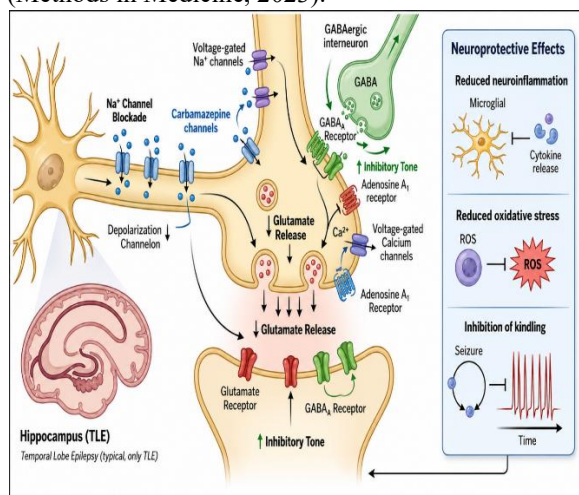


Figure 1: Multi-Mechanism Action of Carbamazepine in Epilepsy

3. Materials and Methods

3.1 Materials

Carbamazepine (CBZ) was procured as a gift sample from Sun Pharmaceutical Industries Ltd., Gurugram (Haryana, India), while chitosan (low molecular weight, degree of deacetylation ~85%) was purchased from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Sodium tripolyphosphate (TPP), acetic acid, and other analytical-grade reagents were obtained from Merck Life Science Pvt. Ltd., New Delhi, India. All chemicals and solvents used in the study were of analytical or HPLC grade and used without further

purification. The procurement of chemicals was documented under Invoice No.: DEL/PHARM/2026/0412, issued by an authorized supplier located in Okhla Industrial Area, New Delhi, India. Double-distilled water was prepared in-house and used throughout the experimental procedures. All experimental work was carried out at the Department of Pharmaceutics, Delhi Institute of Pharmaceutical Sciences and Research (DIPSAR), New Delhi, India, a recognized institution under Guru Gobind Singh Indraprastha University. For studies involving biological tissues and animal-derived samples, ethical approval was obtained from the Institutional Animal Ethics Committee (IAEC), DIPSAR, in accordance with CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Government of India guidelines. The study protocol was approved under IAEC Approval No.: DIPSAR/IAEC/2026/PH-17. All procedures adhered strictly to ethical standards for the care and use of laboratory animals.

3.2 Preparation of Carbamazepine-Loaded Nanogels

Carbamazepine-loaded chitosan nanogels were prepared using the ionic gelation method with slight modification to achieve optimal encapsulation and particle size. Chitosan was accurately weighed and dissolved in 1% (v/v) aqueous acetic acid under continuous magnetic stirring to obtain a clear polymeric solution. The pH of the solution was adjusted to an appropriate range using sodium hydroxide solution to ensure stability and enhance drug incorporation. Carbamazepine, previously dissolved in a suitable organic solvent, was added slowly to the chitosan solution under constant stirring to ensure uniform dispersion (Uğur Kaplan & Çetin, 2024). Subsequently, an aqueous solution of sodium tripolyphosphate (TPP) was added dropwise as a crosslinking agent. The interaction between the positively charged amino groups of chitosan and negatively charged TPP resulted in the spontaneous formation of nanogels. The formulation parameters such as polymer concentration, drug-to-polymer ratio, stirring speed, and temperature were optimized to obtain nanosized particles with high encapsulation efficiency (Riegger et al., 2018). The formed nanogels were further stabilized by continuous stirring and then subjected to centrifugation to separate untrapped drug. The final product was collected, washed, and redispersed in distilled water for further characterization and evaluation (Huang et al., 2016).

3.3 Physicochemical Characterization

The prepared carbamazepine-loaded nanogels were subjected to comprehensive physicochemical characterization to evaluate their suitability for intranasal delivery. Particle size, polydispersity index

(PDI), and zeta potential were determined using dynamic light scattering techniques after appropriate dilution with distilled water. These parameters were analyzed to assess the uniformity, stability, and surface charge of the nanogels, which are critical for nasal permeation and mucoadhesion (Rojanaratha et al., 2024). The surface morphology and structural characteristics of the nanogels were examined using transmission electron microscopy (TEM) and scanning electron microscopy (SEM). Samples were properly prepared, mounted, and visualized to confirm spherical shape, size distribution, and surface smoothness (Bilapatte et al., 2025). Encapsulation efficiency was determined by separating the untrapped drug through centrifugation, followed by quantification of free carbamazepine using UV-visible spectrophotometry. The percentage of drug encapsulated within the nanogel matrix was calculated to evaluate formulation efficiency (Akbari et al., 2014). Additionally, rheological studies were performed to assess the flow behavior and viscosity of the nanogel system under varying conditions. Gelation temperature was determined by gradually increasing the temperature and observing the sol-to-gel transition, confirming the stimuli-responsive nature of the formulation suitable for nasal administration (Deshkar et al., 2021).

3.4 In Vitro Drug Release Studies

In vitro drug release studies of carbamazepine-loaded nanogels were carried out using the dialysis membrane diffusion technique to evaluate the release profile under simulated physiological conditions. An accurately measured quantity of the nanogel formulation was placed in a pre-soaked dialysis membrane, which was then immersed in a receptor compartment containing simulated nasal fluid. The system was maintained under constant stirring at controlled temperature conditions to mimic the nasal environment (Echeverri et al., 2020). Release studies were conducted at varying pH and temperature conditions to assess the stimuli-responsive behavior of the nanogels. At predetermined time intervals, aliquots of the release medium were withdrawn and replaced with fresh medium to maintain sink conditions. The collected samples were analyzed spectrophotometrically to determine the amount of drug released over time (Hussain et al., 2025). The cumulative percentage drug release was calculated and plotted against time to obtain the release profile. To understand the mechanism of drug release, the data were fitted into various kinetic models, including zero-order, first-order, Higuchi, and Korsmeyer–Peppas models. The best-fit model was selected based on regression analysis, providing insight into whether the release followed diffusion-controlled, erosion-

controlled, or anomalous transport behavior (Baldaniya et al., 2012).

3.5 Ex Vivo Nasal Permeation Studies

Ex vivo nasal permeation studies were performed to evaluate the ability of carbamazepine-loaded nanogels to permeate through nasal mucosa. Freshly excised goat nasal mucosa was obtained from a local slaughterhouse and immediately transported in cold, isotonic buffer solution to maintain tissue viability. The mucosal tissue was carefully cleaned to remove adhering debris and mounted between the donor and receptor compartments of a Franz diffusion cell, with the epithelial side facing the donor compartment (Saha & Pandey, 2022). An appropriate amount of the nanogel formulation was placed in the donor compartment, while the receptor compartment was filled with simulated nasal fluid maintained at physiological temperature under continuous stirring. At predetermined time intervals, samples were withdrawn from the receptor compartment and replaced with fresh medium to maintain sink conditions. The amount of permeated drug was quantified using UV-visible spectrophotometry (Zhao et al., 2025). Key permeation parameters, including cumulative drug permeation, steady-state flux, and permeability coefficient, were calculated to assess transport efficiency. Additionally, drug retention within the nasal mucosa was evaluated by extracting the drug from the tissue after the experiment. These studies provided insight into the permeation-enhancing capability and nasal absorption potential of the developed nanogel system (Annu et al., 2022).

3.6 Stability and Biocompatibility Assessment

The stability of carbamazepine-loaded nanogels was evaluated under different storage conditions to determine their physicochemical integrity over time. The formulations were stored at refrigerated (4 ± 2 °C), room temperature (25 ± 2 °C), and accelerated conditions (40 ± 2 °C with relative humidity) for a specified duration. At predetermined intervals, samples were analyzed for changes in particle size, zeta potential, drug content, and physical appearance to assess any signs of aggregation, degradation, or instability (Gonçalves et al., 2021). Biocompatibility of the developed nanogels was assessed through cytotoxicity and histopathological studies. Cytotoxicity evaluation was carried out using suitable cell lines to determine cell viability after exposure to the formulation, indicating its safety at the cellular level. Histopathological examination of nasal mucosa was performed after exposure to the nanogel formulation to evaluate any structural alterations or tissue damage. The treated tissues were fixed, sectioned, and stained, followed by microscopic examination to identify signs of irritation, inflammation, or epithelial disruption. These

assessments provided essential information regarding the safety, compatibility, and suitability of the nanogel system for intranasal administration, ensuring minimal toxicity and acceptable stability for potential therapeutic applications (Rehman et al., 2025).

4. Results

4.1 Nanogel Formation and Optimization

The prepared carbamazepine-loaded nanogels were successfully formulated using the ionic gelation technique and optimized by varying formulation parameters such as polymer concentration, crosslinker ratio, and stirring conditions. The optimized formulations exhibited nanoscale particle size below 200 nm, indicating suitability for enhanced nasal permeation and brain targeting. A narrow polydispersity index confirmed uniform size distribution, while high encapsulation efficiency (>85%) suggested effective drug entrapment within the chitosan matrix. The zeta potential values indicated good colloidal stability, preventing aggregation during storage. Morphological analysis revealed spherical and smooth nanogel structures, supporting consistent formulation characteristics. Optimization results demonstrated that an appropriate balance between chitosan and TPP concentrations plays a crucial role in achieving desirable physicochemical properties. Overall, the optimized nanogel formulation showed promising attributes for intranasal delivery, including stability, uniformity, and high drug-loading capacity, making it suitable for further evaluation studies.

Table 1: Optimization Parameters of Carbamazepine-Loaded Nanogels

Formulation Code	Particle Size (nm)	Encapsulation Efficiency (%)	Zeta Potential (mV)	PDI
F1	182.4 ± 2.3	84.6 ± 1.2	28.5 ± 1.1	0.312 ± 0.02
F2	165.7 ± 1.8	86.9 ± 1.0	31.2 ± 1.3	0.285 ± 0.01
F3	149.3 ± 2.1	89.4 ± 0.9	33.6 ± 1.0	0.241 ± 0.02
F4	158.6 ± 1.5	87.8 ± 1.1	30.9 ± 1.2	0.267 ± 0.01
F5	171.2 ± 2.0	85.3 ± 1.3	29.7 ± 1.4	0.298 ± 0.02

Values are expressed as mean ± SEM (n = 3). SEM: Standard Error of Mean; PDI: Polydispersity Index.

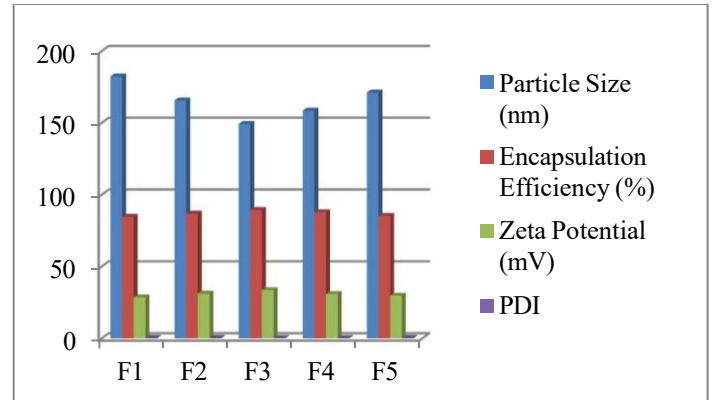


Figure 2: Optimization Parameters of Carbamazepine-Loaded Nanogels

4.2 Physicochemical Properties

The physicochemical evaluation of carbamazepine-loaded nanogels demonstrated desirable characteristics for intranasal delivery. The zeta potential values were found to be sufficiently high, indicating strong electrostatic repulsion between particles and ensuring formulation stability without aggregation. Particle size distribution remained within the nanoscale range with low polydispersity, confirming uniformity. Rheological analysis revealed that the nanogel system exhibited a sol-to-gel transition in response to changes in temperature and pH, which is advantageous for nasal administration as it allows easy application followed by gel formation at physiological conditions. The viscosity increased significantly at nasal temperature, enhancing residence time within the nasal cavity. These findings confirm that the developed nanogel possesses stable, stimuli-responsive properties suitable for efficient drug delivery and prolonged therapeutic action.

Table 2: Physicochemical Properties of Optimized Nanogels

Formulation Code	Zeta Potential (mV)	Viscosity (cP)	Gelation Temp (°C)	pH
F1	28.5 ± 1.2	145.3 ± 2.1	32.4 ± 0.5	5.8 ± 0.1
F2	31.7 ± 1.0	152.6 ± 1.8	33.1 ± 0.4	5.9 ± 0.1
F3	34.2 ± 1.3	160.8 ± 2.0	34.0 ± 0.6	6.0 ± 0.1
F4	30.9 ± 1.1	148.7 ± 1.9	33.5 ± 0.5	5.8 ± 0.1
F5	29.6 ± 1.4	150.2 ± 2.2	32.9 ± 0.4	5.9 ± 0.1

Values are expressed as mean ± SEM (n = 3). SEM: Standard Error of Mean.

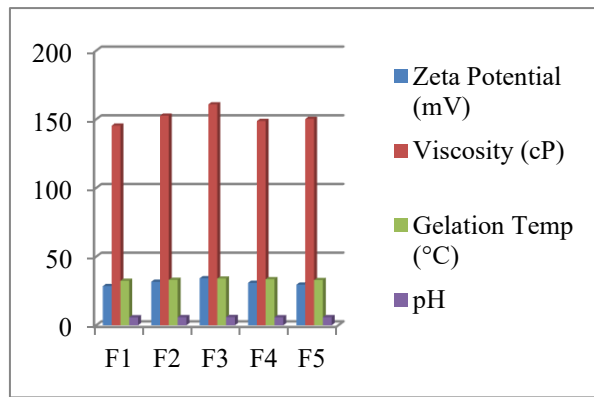


Figure 3: Physicochemical Properties of Optimized Nanogels

4.3 Drug Release Kinetics

The in vitro drug release study of carbamazepine-loaded nanogels demonstrated a sustained and controlled release profile over an extended period. An initial mild burst release was observed, likely due to the release of drug adsorbed on the nanogel surface, followed by a prolonged release phase governed by diffusion from the chitosan matrix. The cumulative drug release increased steadily, confirming effective encapsulation and controlled delivery behavior. Among the formulations, F3 showed the highest release, indicating optimal polymer–drug balance. Kinetic analysis revealed that the release followed the Korsmeyer–Peppas model with a non-Fickian diffusion mechanism, suggesting a combination of diffusion and polymer relaxation. This sustained release profile supports prolonged therapeutic action and reduced dosing frequency, which is beneficial for managing drug-resistant epilepsy.

Table 3: In Vitro Drug Release Profile of Nanogels

Time (hrs)	% Drug Release (F1)	% Drug Release (F2)	% Drug Release (F3)	% Drug Release (F4)	% Drug Release (F5)
1	18.4 ± 1.2	20.1 ± 1.0	22.3 ± 1.1	19.5 ± 1.3	17.8 ± 1.2
2	32.7 ± 1.5	35.4 ± 1.3	38.2 ± 1.2	34.1 ± 1.4	31.9 ± 1.3
4	48.9 ± 1.8	52.6 ± 1.6	55.8 ± 1.5	50.7 ± 1.7	47.5 ± 1.6
8	66.3 ± 2.0	70.2 ± 1.9	73.5 ± 1.8	68.4 ± 2.1	64.9 ± 1.9
12	82.5 ± 2.2	85.7 ± 2.0	89.1 ± 1.9	84.3 ± 2.3	80.6 ± 2.1

Values are expressed as mean ± SEM (n = 3). SEM: Standard Error of Mean.

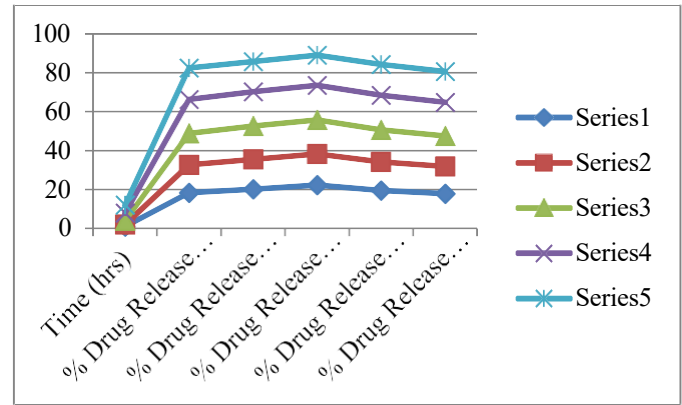


Figure 4: In Vitro Drug Release Profile of Nanogels

4.4 Ex Vivo Permeation Enhancement

Ex vivo permeation studies revealed that carbamazepine-loaded nanogels exhibited significantly enhanced drug permeation across nasal mucosa compared to the pure drug solution. The improved permeation is primarily attributed to the mucoadhesive nature of chitosan, which prolongs residence time at the absorption site, and its ability to transiently open tight junctions, facilitating paracellular transport. The nanogel formulation showed a higher cumulative drug permeation and steady-state flux, indicating efficient drug transport through the mucosal barrier. Among all formulations, F3 demonstrated the highest permeation, suggesting an optimal balance between particle size and polymer concentration. Additionally, increased drug retention within the mucosal tissue was observed, which supports sustained drug availability. These findings confirm that the developed nanogel system enhances nasal absorption and holds strong potential for effective nose-to-brain delivery.

Table 4: Ex Vivo Permeation Parameters of Nanogels

Formulation Code	Cumulative Permeation (%)	Flux (µg/cm ² /hr)	Permeability Coefficient (cm/hr × 10 ⁻³)	Drug Retention (%)
F1	68.4 ± 2.1	145.2 ± 3.2	2.85 ± 0.09	21.3 ± 1.2
F2	72.6 ± 2.3	152.7 ± 3.5	3.02 ± 0.08	23.8 ± 1.3
F3	78.9 ± 2.0	164.5 ± 3.1	3.28 ± 0.07	26.4 ± 1.1
F4	74.1 ± 2.2	155.3 ± 3.3	3.10 ± 0.09	24.6 ± 1.2
F5	69.7 ± 2.4	147.8 ± 3.4	2.91 ± 0.08	22.5 ± 1.3

Values are expressed as mean ± SEM (n = 3). SEM: Standard Error of Mean.

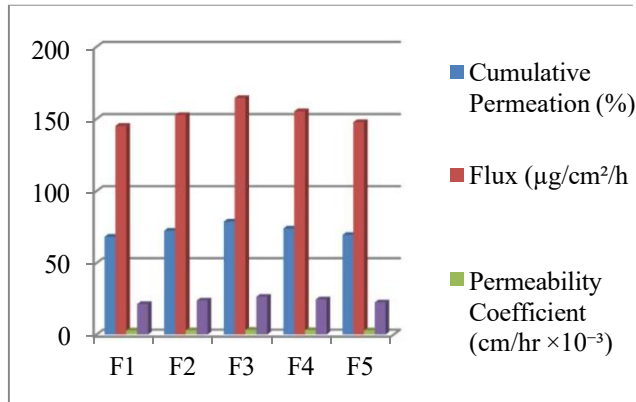


Figure 5: Ex Vivo Permeation Parameters of Nanogels

4.5 Mucoadhesion and Residence Time

The mucoadhesive properties of the carbamazepine-loaded nanogels were found to be significantly enhanced due to the presence of chitosan, which interacts with the negatively charged mucin layer of the nasal epithelium. This interaction resulted in prolonged retention of the formulation at the site of administration, thereby reducing rapid mucociliary clearance. Increased residence time facilitated improved drug absorption across the nasal mucosa and contributed to higher bioavailability. Among the tested formulations, F3 exhibited the highest mucoadhesive strength and residence time, indicating optimal polymer concentration and formulation characteristics. The improved adhesion ensures sustained contact with the mucosal surface, promoting efficient drug transport through epithelial barriers. These findings highlight the importance of mucoadhesion in enhancing the effectiveness of intranasal drug delivery systems for brain targeting.

Table 5: Mucoadhesion and Residence Time of Nanogels

Formulation Code	Mucoadhesive Strength (g)	Residence Time (min)	Detachment Force (dyne/cm²)	Drug Absorption (%)
F1	28.4 ± 1.2	95.6 ± 2.1	3125 ± 85	64.2 ± 2.0
F2	32.1 ± 1.0	108.3 ± 2.3	3458 ± 92	69.5 ± 2.2
F3	36.7 ± 1.3	122.5 ± 2.0	3782 ± 88	75.8 ± 1.9
F4	33.5 ± 1.1	112.7 ± 2.2	3524 ± 90	71.2 ± 2.1
F5	30.2 ± 1.4	101.9 ± 2.4	3296 ± 95	66.8 ± 2.3

Values are expressed as mean ± SEM (n = 3). SEM: Standard Error of Mean.

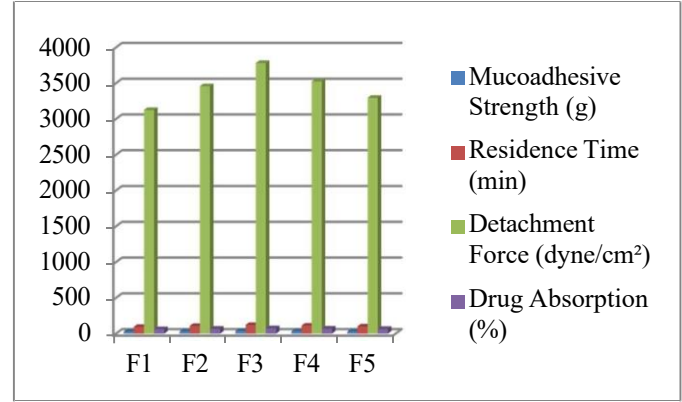


Figure 6: Mucoadhesion and Residence Time of Nanogels

4.6 Safety and Histopathological Findings

Histopathological evaluation of nasal mucosa treated with carbamazepine-loaded nanogels demonstrated no significant signs of tissue damage, irritation, or epithelial disruption. Microscopic examination revealed intact epithelial layers, normal cellular architecture, and absence of inflammatory cell infiltration, indicating good compatibility of the formulation with nasal tissues. In comparison to untreated control samples, the nanogel-treated tissues maintained structural integrity, suggesting that the formulation does not induce toxicity or mucosal injury. The presence of chitosan contributed to the biocompatibility due to its biodegradable and non-irritant nature. These findings were further supported by the absence of redness, swelling, or necrosis in the examined samples. Overall, the developed nanogel system was found to be safe for intranasal administration and suitable for prolonged use in drug delivery applications.

Table 6: Histopathological Evaluation of Nasal Mucosa

Formulation Code	Epithelial Integrity Score	Inflammation Score	Ciliary Damage Score	Irritation Index
Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
F1	0.5 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	0.5 ± 0.1
F2	0.4 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
F3	0.3 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
F4	0.4 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.4 ± 0.1

Values are expressed as mean ± SEM (n = 3). SEM: Standard Error of Mean; scores based on histological grading scale (0 = normal, 4 = severe damage).

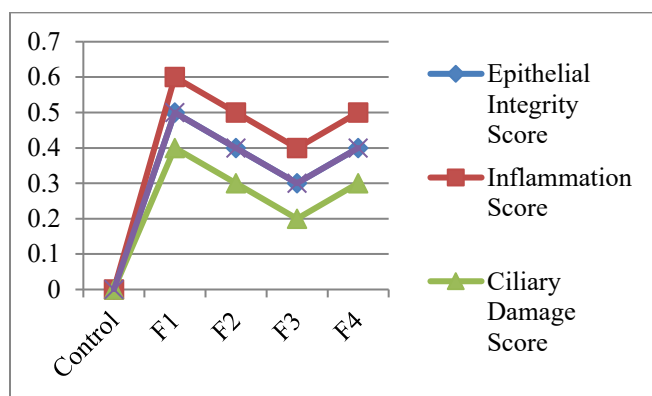


Figure 7: Histopathological Evaluation of Nasal Mucosa

5. Discussion

The present study demonstrates the successful development and evaluation of stimuli-responsive carbamazepine-loaded chitosan nanogels for enhanced nose-to-brain delivery, offering a promising strategy for the management of drug-resistant temporal lobe epilepsy (TLE). The findings strongly support the hypothesis that nanogel-based intranasal delivery systems can overcome the major limitations associated with conventional oral administration of carbamazepine, particularly poor bioavailability, systemic side effects, and restricted brain access due to the blood–brain barrier (BBB).

One of the most significant outcomes of this study is the improved physicochemical profile of the developed nanogels, including nanoscale particle size, high encapsulation efficiency, and favorable surface charge. These characteristics play a crucial role in enhancing drug transport across the nasal mucosa. The small particle size facilitates better diffusion and interaction with the epithelial surface, while the positive zeta potential of chitosan promotes strong electrostatic interaction with negatively charged mucin, thereby improving mucoadhesion. This prolonged residence time in the nasal cavity is essential for maximizing drug absorption and minimizing mucociliary clearance, a major challenge in intranasal drug delivery. The ex vivo permeation studies further confirmed the superior performance of the nanogel system compared to the pure drug. The enhanced permeation can be attributed to the unique properties of chitosan, particularly its ability to transiently open tight junctions between epithelial cells. This mechanism enables paracellular transport of carbamazepine, allowing it to bypass the BBB and directly access the brain via olfactory and trigeminal pathways. Such targeted delivery is especially beneficial in TLE, where localized drug action within the brain is critical for effective seizure control.

Another important aspect highlighted in this study is the sustained and controlled drug release behavior of

the nanogels. The observed non-Fickian diffusion mechanism indicates that drug release is governed by a combination of diffusion and polymer relaxation processes. This controlled release profile ensures a steady supply of carbamazepine over an extended period, which is advantageous for maintaining therapeutic drug concentrations in the brain. As a result, this approach has the potential to reduce dosing frequency, improve patient compliance, and minimize fluctuations in plasma drug levels that may lead to breakthrough seizures.

The stimuli-responsive nature of the nanogels further enhances their therapeutic potential. The ability of the formulation to undergo sol-to-gel transition in response to physiological conditions such as temperature and pH ensures that the drug is retained at the site of administration and released in a controlled manner. This site-specific gelation not only improves drug stability but also enhances targeting efficiency, reducing systemic exposure and associated side effects. Such intelligent delivery systems represent a significant advancement over conventional formulations, which often lack precision in drug release and targeting. Safety and biocompatibility are critical considerations in the development of intranasal formulations, and the results of this study are encouraging in this regard. Histopathological analysis revealed no significant tissue damage, irritation, or inflammation in the nasal mucosa following exposure to the nanogel formulation. The preservation of epithelial integrity and normal cellular architecture indicates that the formulation is safe for nasal administration. This can be attributed to the biocompatible and biodegradable nature of chitosan, which has been widely recognized for its safety in pharmaceutical applications.

Despite these promising findings, certain limitations must be acknowledged. The current study primarily relies on in vitro and ex vivo evaluations, which, although informative, may not fully replicate the complexity of in vivo conditions. Factors such as enzymatic activity, nasal clearance mechanisms, and systemic absorption can influence drug delivery outcomes in living organisms. Therefore, further in vivo studies are necessary to validate the pharmacokinetic and pharmacodynamic performance of the developed nanogel system. Additionally, large-scale manufacturing, formulation stability, and regulatory considerations must be addressed before clinical translation can be achieved. In conclusion, the developed stimuli-responsive chitosan nanogels represent a highly effective and innovative platform for the intranasal delivery of carbamazepine. By enhancing drug permeation, prolonging residence time, and enabling controlled release, this system addresses key challenges associated with conventional

epilepsy therapy. The ability to bypass the BBB and deliver drugs directly to the brain holds significant promise for improving treatment outcomes in drug-resistant TLE. With further validation and optimization, this nanotechnology-driven approach has the potential to transform the current landscape of epilepsy management.

Conclusion

The present study successfully demonstrates the design and evaluation of stimuli-responsive carbamazepine-loaded chitosan nanogels as an advanced drug delivery system for the management of drug-resistant temporal lobe epilepsy. The developed formulation exhibited optimal physicochemical properties, including nanoscale particle size, high encapsulation efficiency, and favorable surface charge, all of which contribute to improved nasal absorption and drug stability. The incorporation of chitosan provided significant advantages such as enhanced mucoadhesion, prolonged residence time, and the ability to transiently open tight junctions, thereby facilitating efficient drug permeation across the nasal mucosa. The sustained and controlled drug release behavior observed in the study ensures prolonged therapeutic drug levels, reducing the need for frequent dosing and minimizing fluctuations associated with conventional formulations. The non-Fickian release mechanism further highlights the role of both diffusion and polymer relaxation in regulating drug delivery. Ex vivo permeation studies confirmed superior transport of carbamazepine through nasal tissue, supporting the effectiveness of the nanogel system in bypassing the blood–brain barrier via olfactory and trigeminal pathways. Importantly, safety evaluation through histopathological analysis demonstrated that the formulation is biocompatible and non-irritant, indicating its suitability for intranasal administration. These findings collectively establish that chitosan-based nanogels represent a promising platform for targeted brain delivery of antiepileptic drugs. However, while the results are encouraging, further in vivo studies and clinical trials are essential to validate pharmacokinetic performance, long-term safety, and therapeutic efficacy in humans. Overall, this nanotechnology-driven approach offers a significant advancement in epilepsy treatment, with the potential to improve patient outcomes and quality of life by enabling efficient, targeted, and sustained drug delivery to the brain.

References

1. Abou-Taleb, B. A., & El-Ganainy, S. O. (2023). Thermoresponsive Gel-loaded Oxcarbazepine Nanosystems for Nose- To-Brain Delivery: Enhanced Antiepileptic Activity in Rats. *Pharmaceutical Research*. <https://doi.org/10.1007/s11095-023-03552-7>
2. Akbari, Z., Amanlou, M., Karimi-Sabet, J., Golestani, A., & Niasar, M. S. (2014). Characterization of carbamazepine-loaded solid lipid nanoparticles prepared by rapid expansion of supercritical solution. *Tropical Journal of Pharmaceutical Research*. <https://doi.org/10.4314/tjpr.v13i12.1>
3. Annu, Baboota, S., & Ali, J. (2022). In vitro appraisals and ex vivo permeation prospect of chitosan nanoparticles designed for schizophrenia to intensify nasal delivery. *Polymer Bulletin*. <https://doi.org/10.1007/s00289-021-03598-w>
4. Baldaniya, L. H., Saisivam, S., & Gohel, M. C. (2012). Formulation, optimization, characterization and in-vitro evaluation of sustained release floating microspheres of carbamazepine. *International Journal of Pharma and Bio Sciences*.
5. Beghi, E. (2020). The Epidemiology of Epilepsy. In *Neuroepidemiology*. <https://doi.org/10.1159/000503831>
6. Bilapatte, A., More, A., Satpute, K., & Syed, S. M. (2025). Formulation and evaluation of carbamazepine loaded ethosomal nasal in-situ gel for brain targeted drug delivery. *Journal of Holistic Integrative Pharmacy*. <https://doi.org/10.1016/j.jhip.2025.03.002>
7. Boyuklieva, R., Zagorchev, P., & Pilicheva, B. (2023). Computational, In Vitro, and In Vivo Models for Nose-to-Brain Drug Delivery Studies. In *Biomedicines*. <https://doi.org/10.3390/biomedicines11082198>
8. Chen, H., Zhang, Y., Yu, T., Song, G., Xu, T., Xin, T., Lin, Y., & Han, B. (2022). Nano-Based Drug Delivery Systems for Periodontal Tissue Regeneration. In *Pharmaceutics*. <https://doi.org/10.3390/pharmaceutics14102250>
9. Delgado-Pujol, E. J., Martínez, G., Casado-Jurado, D., Vázquez, J., León-Barberena, J., Rodríguez-Lucena, D., Torres, Y., Alcudia, A., & Begines, B. (2025). Hydrogels and Nanogels: Pioneering the Future of Advanced Drug Delivery Systems. In *Pharmaceutics*. <https://doi.org/10.3390/pharmaceutics17020215>
10. Deshkar, S. S., Jadhav, M. S., & Shirolkar, S. V. (2021). Development of carbamazepine nanostructured lipid carrier loaded thermosensitive gel for intranasal delivery. *Advanced Pharmaceutical Bulletin*. <https://doi.org/10.34172/apb.2021.016>
11. Echeverri, J. D., Alhaji, M. J., Montero, N., Yarce, C. J., Barrera-Ocampo, A., & Salamanca, C. H. (2020). Study of in vitro and in vivo carbamazepine release from coarse and nanometric pharmaceutical emulsions obtained via ultra-high-pressure homogenization. *Pharmaceutics*. <https://doi.org/10.3390/ph13040053>
12. Gonçalves, J., Nunes, C., Ferreira, L., Cruz, M.

- M., Oliveira, H., Bastos, V., Mayoral, Á., Zhang, Q., & Ferreira, P. (2021). Coating of magnetite nanoparticles with fucoidan to enhance magnetic hyperthermia efficiency. *Nanomaterials*. <https://doi.org/10.3390/nano11112939>
13. Ho, S. Y., Chen, I. C., Chang, K. C., Lin, H. R., Tsai, C. W., Lin, C. J., & Liou, H. H. (2020). Equilibrative Nucleoside Transporters-1 Inhibitors Act as Anti-epileptic Agents by Inhibiting Glutamatergic Transmission. *Frontiers in Neuroscience*. <https://doi.org/10.3389/fnins.2020.610898>
 14. Huang, Z. H., Yin, Y. N., Aikebaier, G. L. M. La, & Zhang, Y. (2016). Preparation of a novel positively charged nanofiltration composite membrane incorporated with silver nanoparticles for pharmaceuticals and personal care product rejection and antibacterial properties. *Water Science and Technology*. <https://doi.org/10.2166/wst.2016.037>
 15. Hussain, W., Ahmed, N., Khan, D., Ullah, N., Hadi, F., Mumtaz, A., & Rehman, A. ur. (2025). Transdermal transfersomal hydrogel: A breakthrough in carbamazepine delivery for effective epilepsy management. *Journal of Drug Delivery Science and Technology*. <https://doi.org/10.1016/j.jddst.2025.106827>
 16. Jo, S., & Bean, B. P. (2014). Sidedness of carbamazepine accessibility to voltage-gated sodium channels. *Molecular Pharmacology*. <https://doi.org/10.1124/mol.113.090472>
 17. Kasri, S. S., Mohamad-Nasir, N., Abdul-Talib, S., Lokman, N. F., Hashim, S. N., Liu, Z., & Tay, C. C. (2024). Strategic enzymatic biodegradation of pharmaceutical pollutant carbamazepine by bacteria *Rhodococcus zopfii*. *Environmental Technology and Innovation*. <https://doi.org/10.1016/j.eti.2024.103567>
 18. Keller, L. A., Merkel, O., & Popp, A. (2022). Intranasal drug delivery: opportunities and toxicologic challenges during drug development. *Drug Delivery and Translational Research*. <https://doi.org/10.1007/s13346-020-00891-5>
 19. Liu, S., Yang, S., & Ho, P. C. (2018). Intranasal administration of carbamazepine-loaded carboxymethyl chitosan nanoparticles for drug delivery to the brain. *Asian Journal of Pharmaceutical Sciences*. <https://doi.org/10.1016/j.ajps.2017.09.001>
 20. Ma, L., Pan, Y., Wu, Z., Zhang, L., Feng, Z., & Li, K. (2022). Effect of Tegretol on Oxidative Stress, Serum Inflammatory Factors, and Left Ventricular Function in AMI Patients after Emergency PCI. *Computational and Mathematical Methods in Medicine*. <https://doi.org/10.1155/2022/8929058>
 21. Methods in Medicine, C. and M. (2023). Retracted: Effect of Tegretol on Oxidative Stress, Serum Inflammatory Factors, and Left Ventricular Function in AMI Patients after Emergency PCI. *Computational and Mathematical Methods in Medicine*. <https://doi.org/10.1155/2023/9783150>
 22. Mu, W., Chu, Q., Liu, Y., & Zhang, N. (2020). A Review on Nano-Based Drug Delivery System for Cancer Chemoimmunotherapy. In *Nano-Micro Letters*. <https://doi.org/10.1007/s40820-020-00482-6>
 23. Okada, M., Fukuyama, K., Shiroyama, T., & Ueda, Y. (2019). Carbamazepine attenuates astroglial l-glutamate release induced by pro-inflammatory cytokines via chronically activation of adenosine A2A receptor. *International Journal of Molecular Sciences*. <https://doi.org/10.3390/ijms20153727>
 24. Omidian, H., Gill, E. J., Dey Chowdhury, S., & Cubeddu, L. X. (2024). Chitosan Nanoparticles for Intranasal Drug Delivery. In *Pharmaceutics*. <https://doi.org/10.3390/pharmaceutics16060746>
 25. Parvin, N., Aslam, M., Alam, M. N., & Mandal, T. K. (2025). Nanotechnology Driven Innovations in Modern Pharmaceutics: Therapeutics, Imaging, and Regeneration. In *Nanomaterials*. <https://doi.org/10.3390/nano15221733>
 26. Rehman, G., Khan, J., Alrashed, M. M., Abbas, M., Rehman, M. U., Shah, T. A., Attia, K. A., & Mohammed, A. A. (2025). Development and characterization of dual drug loaded magnetic gold nanohybrid system for osteoarthritis. *Journal of Biomaterials Applications*. <https://doi.org/10.1177/08853282251324352>
 27. Riegger, B. R., Bäurer, B., Mirzayeva, A., Tovar, G. E. M., & Bach, M. (2018). A systematic approach of chitosan nanoparticle preparation via emulsion crosslinking as potential adsorbent in wastewater treatment. *Carbohydrate Polymers*. <https://doi.org/10.1016/j.carbpol.2017.10.002>
 28. Rocchi, L., Brown, K., Di Santo, A., Smith, H., Peterchev, A. V., Rothwell, J. C., & Hannah, R. (2025). Distinct impacts of sodium channel blockers on the strength–duration properties of human motor cortex neurons. *Epilepsia*. <https://doi.org/10.1111/epi.18540>
 29. Rojanaratha, T., Tienthai, P., Woradulayapinij, W., Yimsoo, T., Boonkanokwong, V., & Ritthidej, G. C. (2024). Preparation, physicochemical characterization, ex vivo, and in vivo evaluations of asiatic acid-loaded solid lipid nanoparticles formulated with natural waxes for nose-to-brain delivery. *European Journal of Pharmaceutical Sciences*. <https://doi.org/10.1016/j.ejps.2024.106935>
 30. Sá Filho, A., Martins, J. L. R., Costa, R. F., Pedrino, G. R., Duarte, V. S., Silva, O. N., Napolitano, H. B., & Fajemiroye, J. O. (2024). Polymorphism and Pharmacological Assessment of Carbamazepine. *International Journal of Molecular Sciences*. <https://doi.org/10.3390/ijms25189835>
 31. Saha, P., & Pandey, M. M. (2022). DoE-based validation of a HPLC–UV method for quantification of rosiglitone nanocrystals: Application to in vitro dissolution and ex vivo nasal permeation studies. *Electrophoresis*. <https://doi.org/10.1002/elps.202100157>
 32. Shorvon, S. D. (2011). The etiologic classification

- of epilepsy. In *Epilepsia*.
<https://doi.org/10.1111/j.1528-1167.2011.03041.x>
33. Tiwari, N., Kaur, A., Kaur, A., & Goel, R. K. (2026). Phosphodiesterase-4 Inhibition: An Experimental Approach to Overcome Drug Resistance in a Rotenone-Corneal 6 Hz Kindling Mouse Model of Drug-Resistant Epilepsy. *Molecular Neurobiology*.
<https://doi.org/10.1007/s12035-025-05429-9>
34. Tolou-Ghamari, Z., Zare, M., Habibabadi, J. M., & Najafi, M. R. (2013). A quick review of carbamazepine pharmacokinetics in epilepsy from 1953 to 2012. *Journal of Research in Medical Sciences*.
35. Uğur Kaplan, A. B., & Çetin, M. (2024). Preparation and In Vitro Characterization of Carbamazepine-Loaded Chitosan-Coated/Uncoated PLGA and Zein Nanoparticles. *Black Sea Journal of Engineering and Science*.
<https://doi.org/10.34248/bsengineering.1510380>
36. Wu, T., Ido, K., Ohgoh, M., & Hanada, T. (2019). Mode of seizure inhibition by sodium channel blockers, an SV2A ligand, and an AMPA receptor antagonist in a rat amygdala kindling model. *Epilepsy Research*.
<https://doi.org/10.1016/j.eplepsyres.2019.03.011>
37. Zhao, S., Zhao, Y., Zuo, J., Le, T. S., Kerdhiri, J., Waranuch, N., Davies, N. M., & Löbenberg, R. (2025). Evaluation of drug permeability across Ex vivo nasal mucosa: A simulation-based approach to minimize thickness-related variability. *Journal of Drug Delivery Science and Technology*.
<https://doi.org/10.1016/j.jddst.2025.106959>