

Formulate Chitosan Coated Optimized Nanoliposomes Of Lamotrigine Through Intranasal Route For Management Of Epilepsy

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Abstract

One of the most telling symptoms of this disorder is widespread electrical abnormalities throughout the brain. The new formulation may be given as a once-daily formulation, it is helpful in treating epilepsy since it lessens the LTG-SOL's drawbacks in terms of how often it must be taken, how much it must be taken, the side effects it causes, and how much it costs. Yet, the proof of concept established by this research requires further validation in higher animals and human participants.

Keywords: Lamotrigine; nose-to-brain delivery; PLGA nanoparticles; epilepsy

INTRODUCTION

Seizures, brought on by the aberrant electrical activity characteristic of epilepsy, may affect any region of the brain. Over 50 million individuals throughout the world are affected by this condition, which has neurological, cognitive, psychological, and social implications (Devinsky et al., 2018). Epilepsy prevalence and incidence rates are estimated differently between countries. Although the disease is more prevalent in low- and middle-income nations than in high-income ones, it disproportionately affects younger people (Fiest et al., 2017). Nonetheless, deaths are uncommon. Epilepsy was shown to contribute to fatalities from both accidental and self-inflicted causes (Thurman et al., 2017). These findings demonstrate that patients have mental health consequences as a result of the disease's chronic nature.

Lamotrigine (LAM) is a second-generation phenyltriazine anti-epileptic medication that is only accessible as tablets at the moment. Several *in vivo* studies have used it, although they have often been conducted to assess its immunomodulatory function, bioactivation, or formation of gastroretentive matrix tablets, chewable/dispersible tablets, or intravenous (iv) nanoformulation. The pharmacokinetic features of the API were investigated in another investigation in which LAM was administered intranasally.

The FDA has approved many tablet formulations of LTG, including those with immediate release, prolonged release, orally disintegrating, and chewable formats. However, Because of its low solubility, untargeted administration, failure to cross the BBB, substantial hepatic metabolism, and dietary influence on pharmacokinetics, oral LTG cannot fulfill its full therapeutic potential and is not suitable for use in long-term epilepsy treatment. Solid dispersion and nanosuspension are only two of the many LTG formulations that have been tried and evaluated. The oral route is favored for LTG in the therapy of epilepsy, despite the fact that these methods have been effective in enhancing solubility, lowering negative side effects associated with highdose, and preventing administration to comatose patients. Nevertheless, this approach is intrusive and must be

administered by a trained medical specialist, thus it is reserved for extreme circumstances only. To far, no LTG formation has shown both site-specific targeting and high bioavailability in the brain, despite extensive research. In light of these constraints, it is of scientific interest to work on new LTG formulations with alternative modes of administration. The blood-brain barrier (BBB) makes brain drug distribution difficult. The intranasal method offers a noninvasive option for CNS drug delivery since it avoids crossing the blood-brain barrier. Nasal administration is preferred because it improves patient accessibility, compliance, initiation of action, avoidance of first pass metabolism, absorption rate, and systemic exposure. As compared to intravenous distribution, The thermoreversible features of LTGnasal gel, developed by Serralheiro and coworkers, enable for a more controlled release of the medication and a sustained elevation of its concentration in the brain. Colloidal particles of 1 to 1000 nm in size, nanoparticles are often fabricated from synthetic or semi-synthetic polymers. Combining intranasal administration with nanotechnology methods to package the medication may improve brain targeting, decrease the dose, and increase the bioavailability of the treatment in the brain, according to some current studies.

The results demonstrated improved rotigotine targeting efficacy and brain bioavailability. For the treatment of depression, Haque et al. created a formulation of alginate nanoparticles loaded with Venlafaxine and delivered it intranasally. Researchers found that alginate nanoparticles were more effective than intranasal and intravenous Venlafaxine solutions for brain uptake, pharmacokinetics, and pharmacodynamics. For the treatment of epilepsy, For intracranial injection, The LTG nanostructured-lipid-carriers were created and improved by Alam et al. It has been reported that a greater concentration of LTG is achieved in the brain after intranasal (IN) injection of nanostructured-lipid-carriers loaded with LTG compared to IN and oral drug solution in rats. To enhance medication delivery to the hippocampus, Yu and colleagues created an intranasal mixed micellar system based on mPEG-PLA/TPGS. The findings demonstrated that the formulation improved nasal LTG absorption and decreased LTG brain efflux.

LITERATURE REVIEW

Argelia Rosillo-de la Torre, et al. (2014), The neurological condition known as epilepsy is quite prevalent. It's a big issue for public health since it's linked to worse overall health and quality of life. There is a substantial proportion of individuals with epilepsy (35-40%) who are resistant to pharmacotherapy, despite the huge number of existing and the continuous development of various novel antiepileptic medications (AEDs). Overexpression of multidrug resistance proteins like Pglycoprotein on the endothelium of the blood brain barrier is thought to be one explanation for pharmacoresistance in epilepsy. This poses a problem for the efficient transport and concentration of AEDs in the brain. Epilepsy surgery and neuromodulation are two treatment methods that have been shown to be effective in managing pharmacoresistant epilepsy. The bad news is that not every patient can benefit from these treatments. In individuals with pharmacoresistant epilepsy, nanotechnology provides an appealing technique to circumvent the restricted brain access of AEDs. This paper provides a summary of the evidence that backs up this claim.

P. K. Gangurde; et al. (2019), Background: Pharmaceutical research in the medical profession is conducted with the ultimate goal of enhancing the patient's standard of living. Since antiepileptic medications have trouble crossing the blood brain barrier, many people all over the globe suffer from the neurological condition epilepsy. Thus, it is important to develop new methods of medication administration to circumvent the drawbacks of traditional treatment. This review will introduce and explain the significance of nasal delivery of lamotrigine to the brain. How drugs are carried from the nose to the brain, and how nasal absorption might be enhanced. Lamotrigine is currently only available for oral delivery. This article focuses emphasis on the nasal route of drug delivery, a somewhat uncharted territory for the targeting of lamotrigine to the brain. The majority of the

strategies attempted to administer the nanoparticles orally or intravenously. The future of medication delivery to specific areas of the brain through the nasal route has been considered in this article. We conclude that delivering lamotrigine directly to the brain via the nasal passages is a potential delivery technique that might also benefit the treatment of other poorly accessible medicines for psychiatric diseases.

Anjali P B, Jawahar Natarajan, Arun Radhakrishnan (2019), One of the most widespread and potentially devastating central nervous system (CNS) diseases is epilepsy. Epilepsy affects over 70 million individuals worldwide, with an annual incidence rate ranging from 34,000 to 76,000 new cases per 100,000. Patients with epilepsy can face hidden bias, exclusion, and discrimination in social and professional contexts. People with epilepsy often suffer from emotional distress, social isolation, missed career opportunities, and personal devastation. Consequences of epilepsy include a worse quality of life for the patient, an increased risk of injury or death, and a monetary and educational burden for the family. Damage, suffocation, depression, anxiety, and a high suicide risk are only some of the many physical and emotional complications that are linked to epilepsy. Unchecked epileptic seizures and the progression of epilepsy provide a greater risk of horrific outcomes and impair memory, cognition, and endocrine ability. The goal is to eliminate seizures by delivering the drug to the brain in sufficient quantities to do so without producing unintended side effects. Thirty percent of people with epilepsy will never be cured because the amount needed to control seizures causes unwelcome side effects. This overview focuses on novel strategies for improving upon the limitations of conventional epilepsy care.

C. Costa, et al. (2019), Severe diseases like epileptic seizures and anxiety crises need prompt, efficient therapy that focuses on the brain. While they may be given orally, intravenously, or rectally in an emergency, the brain bioavailability of current antiepileptics and anxiolytics is minimal. This is associated with the little degree to which these medicines are able to cross the blood-brain barrier (BBB). The development of strategies to significantly increase the brain bioavailability of these drugs, coupled with a simple and safe administration by patients, is urgently needed despite the progress made in treating and preventing epileptic seizures and anxiety crises. As a result, intranasal and nasal drug administration has been offered as a way to bypass the BBB on the way to the central nervous system.

Patrcia Severino, et al. (2018), Poor medication absorption, limited brain exposure, rapid metabolism and elimination, high doses, and unwanted side effects may be mitigated by using this strategy, nanotechnology-based nasal delivery devices have received attention. Non-invasiveness, convenience, accessibility, a permeable epithelial barrier, and highly vascularized tissue are the key advantages of intranasal (IN) administration. Yet, in order to guarantee adequate release, it is essential to determine how the materials used interact with the nasal biological environment.

Materials and Methods

IPCA Laboratories and Evonik Healthcare generously provided free samples of LTG and PLGA, respectively. Sigma-Aldrich provided the poloxamer 407, while the other chemicals were acquired from Merck Scientific. These chemicals included acetone, potassium dihydrogen phosphate, sodium chloride, methanol, and acetonitrile. Himedia Laboratories Pvt. Ltd. was contacted to get dialysis membranes and polycarbonate syringe filters.

Preparation of LTG-PNPs

The modified aqueous phase was being sonicated at high speeds in a Probe sonicator, the organic phase was introduced very slowly. The liquid was then heated to 25 °C while being agitated slowly on a magnetic stirrer in order to evaporate the acetone. A 32-factorial design was used to find the optimal values for key material characteristics, such as PLGA content and surfactant concentration.

Table 1 displays the results of an ionotropic gelation procedure in which eight batches of LT microspheres were produced using different drug-polymer CH and crosslinking agent glutaraldehyde concentrations. To get its cationic solution, CH was dissolved in an aqueous 1% v/v acetic acid solution. Cationic solution from CH was used to dissolve the LT. The aforesaid solution was then gradually added to the polyanionic glutaraldehyde solution at a rate of 30 drops per minute from a height of 5 cm while being continuously stirred at 800 rpm with a mechanical stirrer. Similar to the drug-filled microspheres, blank microspheres were manufactured using the same technique. Optimizing LT microspheres required experimenting with varying drug, polymer, and crosslinking agent concentrations.

Table 1: Composition of different batches of lamotrigine loaded chitosan microspheres

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Lamotrigine (mg)	100	100	100	100	200	300	100	100
Chitosan (mg)	100	200	300	400	100	100	200	200
Glutaraldehyde (ml)	2	2	2	2	2	2	3	5

Entrapment efficiency

The suspension of nanoparticles was centrifuged at 4 °C, 20,000 rpm, for 20 minutes. The supernatant was collected after appropriate dilution, and HPLC was used to detect the presence of free LTG. The % EE was determined using the formula:

$$\text{Entrapment efficiency (\%)} = \left[\frac{(\text{Total amount of drug} - \text{Amount of free drug})}{\text{Total amount of drug}} \right] \times 100$$

HPLC was used to calculate LTG. A 25 C Grace Smart C18 column was used for the LTG separation. At a flow rate of 1 ml/min, an isocratic combination of 25% methanol, 25% acetonitrile, and 50% phosphate buffer (pH 7.0) served as the mobile phase. The estimated wavelength of LTG was 225 nm, and its retention duration was 5.9 minutes. 15-50 lg/ml was the calibrated range.

The Zetasizer Nano ZS was used to determine the average particle size, f-potential, and PDI of the LTG-PNPs through the dynamic light scattering method. Every single reading was taken at a constant 25 degrees Celsius and 137 degrees. The polydispersity index (PDI) was calculated to assess the dispersion's uniformity or diversity; a value of 0.5 indicates a monodisperse, uniform population of nanoparticles.

To track LTG secretion, a dialysis bag approach was used. Two milliliters of LTG-SOL/LTG-PNPs were added to the dialysis bag, and the release medium was maintained at 37 °C with steady stirring. Removal of the receptor compartment and subsequent replacement with release media (5 ml aliquots) occurred at 1, 2, 3, 4, 6, 8, 12, and 24 hours. HPLC was used to get an estimate once the samples were properly diluted.

The thermograms of LTG and LTG-PNPs were recorded using a differential scanning calorimeter. In order to scan the temperature, range from 25 to 300 degrees Celsius, ten milligram samples were crimped in empty aluminum pie plates. At a rate of 10 degrees Celsius per minute, nitrogen was purged at a rate of 20 milliliters per minute. The thermograms of LTG and LTG-PNPs were recorded using a differential scanning calorimeter. In order to scan

the temperature, range from 25 to 300 degrees Celsius, ten milligram samples were crimped in empty aluminum pie plates. At a rate of 10 degrees Celsius per minute, nitrogen was purged at a rate of 20 milliliters per minute. The nasal mucosa from a goat was put on a Franz diffusion cell to simulate the ex vivo environment. There was 1 ml of LTG-SOL/LTG-PNPs in the donor chamber. The receptor chamber was filled with 20 mL of a 37°C, 2°C methanolic phosphate buffer (pH 6.4) that was constantly being stirred. The receptor chamber was sampled at regular intervals for analysis. The data is presented in the form of Mean SD. The SPSSVR statistical package was used to conduct a one-way analysis of variance (ANOVA) and t-test for independence. The cutoff for significance was set at p .05.

RESULTS AND DISCUSSION

The emulsification-solvent evaporation method was used to create LTG-PNPs. A 32-factorial layout was used for the optimization (data not shown). Table 2 displays the findings and the improved formulation parameters. Mean particle size for LTG-PNPs was 170 nm, particle size distribution index (PDI) was 0.19, and zeta potential was 16.60 mV. Because of their small size (200nm), LTG-PNPs administered through the nose are well-suited for brain targeting.

Poor aqueous LTG solubility led to partial release of 42% (Figure 1) from LTG-SOL after 24 hours. Almost total release (94.5%) from LTG-PNPs may be due to their nanometric size and the inclusion of Poloxamer 407, which promotes permeability, wetting, solubilization, and dissolution by producing holes in the matrix and accelerates LTG release.

Table 2. Parameter optimization for the preparation of LTG-PNPs

Parameter	Optimized value
Amount of PLGA (mg)	82
Concentration of Poloxamer 407 (%)	0.75
% EE	71.3 ± 2.0
Particle size (nm)	170.0 ± 2.8
% CDR at 24 h	94.5 ± 4.2
PDI	0.191 ± 0.035
Zeta potential (mV)	-16.60 ± 2.96

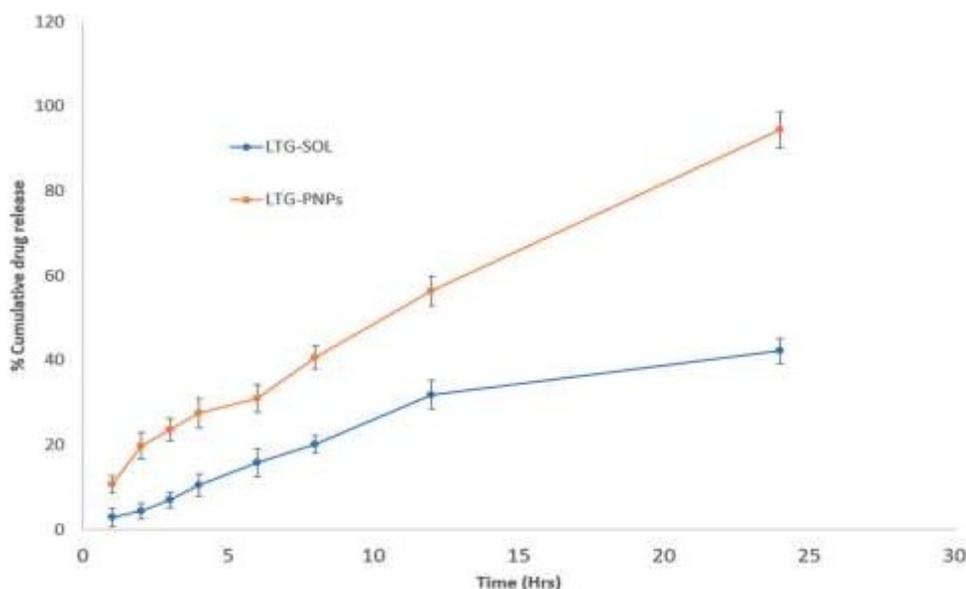


Figure 1. In-vitro release profiles of LTG-SOL & LTG-PNPs

The release profile of LTG-PNPs was biphasic, with the first 20% being released rapidly within the first 2 hours and the remainder 75-80% being released slowly over the next 22 hours. Consistent with the conclusions of previous researchers. With a large amount of exposed surface area compared to its total volume, Both free LTG in the external phase and LTG adsorption on the surface of polymeric nanoparticles have been proposed as first release triggers. The sluggish diffusion of LTG into the aqueous medium was the result of its full and homogenous encapsulation inside the matrix of PLGA, which led to the prolonged release [44,45]. The initial burst release and extended release of LTG-PNPs allow doctors to obtain loading and maintenance dosages. This twofold release mechanism is helpful because it reduces the pharmacokinetic variability of LTG by stabilizing plasma concentrations.

Kinetics of LTG-PNPs were well-fit by both the Higuchi and Korsmeyer-Peppas models ($R^2 > .99$). The n value of 0.6298 suggests anomalous transport, which is likely a combination of polymer erosion/degradation and drug diffusion mechanisms.

Figure 2(A) shows that the melting point of LTG, implying its crystalline form, corresponds to a pronounced endothermic peak at 217.02 C. In the LTG-PNPs, The disappearance of the robust endothermic peak suggests that LTG has changed from its crystalline to amorphous state upon entering the PLGA-supplied lipid matrix. (Figure 2(B)). These findings corroborate those found by other writers.

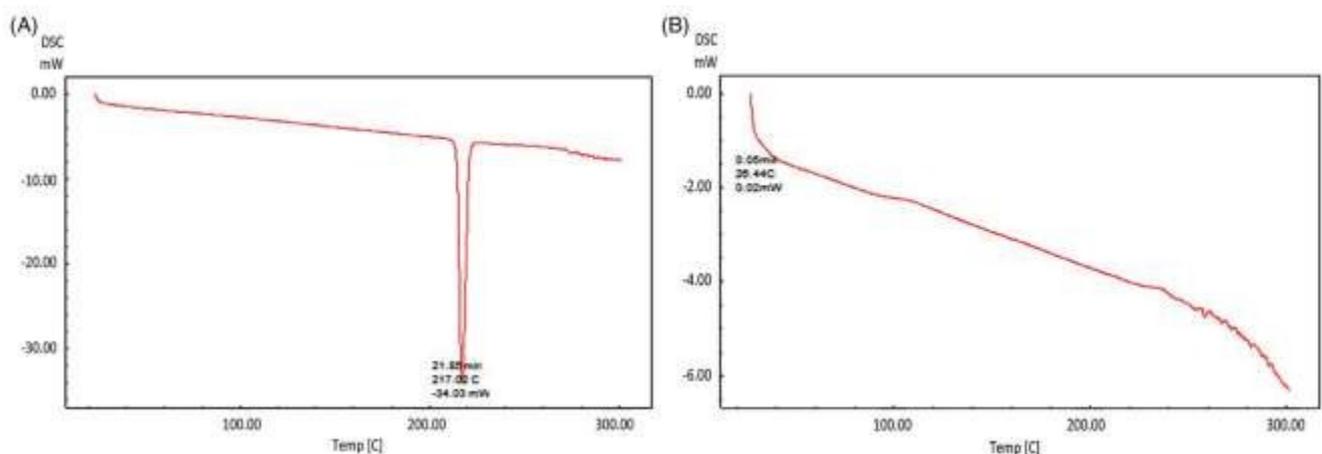


Figure 2. DSC Thermogram of (A) Pure LTG (B) LTG-PNPs

Figure 3(A) displays distinct and powerful peaks for LTG at 8.2, 10.2, 13.1, 25.9, and 27.9 2 θ , all of which are consistent with a crystalline structure. A lack of strong intensity peaks and the presence of a dome-shaped area in the XRD pattern of PLGA (Figure 3(B)) are characteristic of amorphous materials. Poloxamer 407 has a distinct and prominent peak, as seen in Figure 3(C). The XRD analysis of LTG-PNPs revealed that the molecularly dispersed LTG disappears as sharp and strong peaks, demonstrating its amorphous character. (Figure 3(D)). Seju also saw something similar.

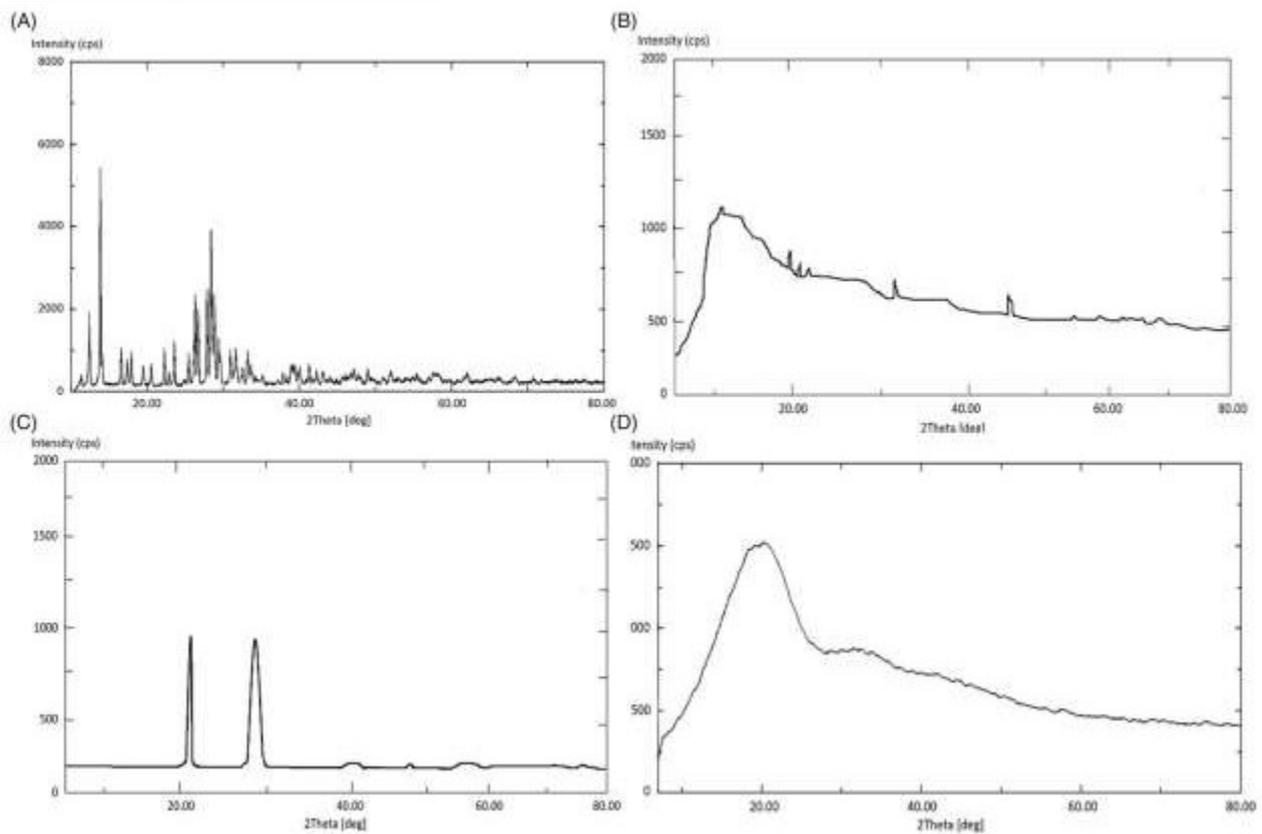


Figure 3. X-ray diffractogram of (A) pure LTG (B) PLGA (C) Poloxamer 407 (D) LTG-PNPs.

Ex-vivo studies

We measured permeability coefficients of $1.316 (0.27) \times 10^3$ cm/h for LTG-SOL and $3.264 (0.33) \times 10^3$ cm/h for LTG-PNPs. Drug penetration was 2.5 times greater in LTG-PNPs than in LTG-SOL (Figure 4). This may be explained by the nanometric size of LTG-PNPs and the presence of Poloxamer 407, a well-known permeability enhancer, on the surface of nanoparticles. Hydrogen bonds are formed between Poloxamer 407 and the nasal mucosa, allowing it to cling to the tissue. In addition to facilitating LTG permeability, Poloxamer 407 serves as a P-gp (efflux pump) inhibitor (P-gp substrate).

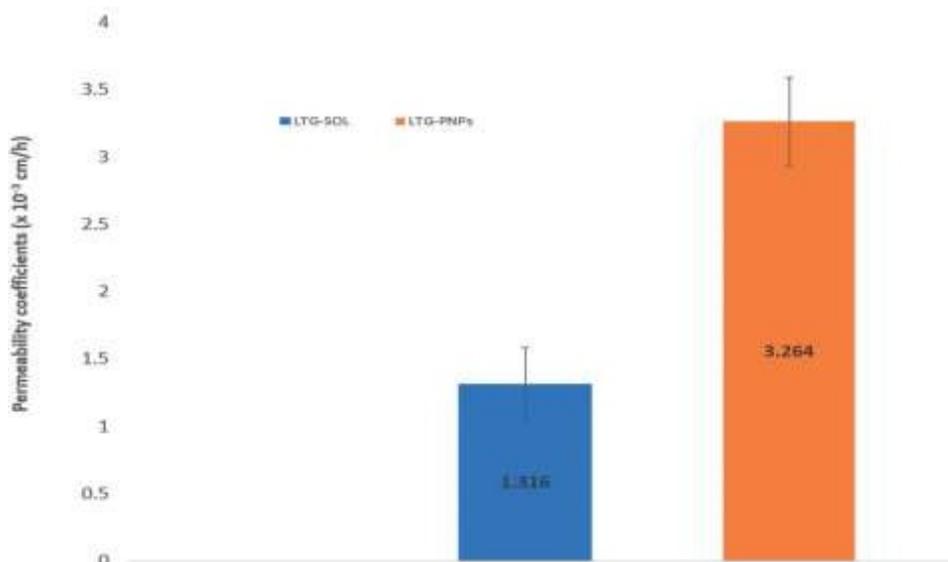


Figure 4. Goat nasal mucosal penetration of LTG-SOL and LTG-PNPs in an ex vivo model

Physicochemical characterization of microspheres

Physicochemical property and mucociliary clearance primarily influence nasal bioavailability. Because of their ability to delay mucociliary clearance and increase contact duration with nasal mucosa, CH microspheres have unique benefits in this setting. Table 3 displays the size distribution of several microspheres. Microsphere particle size is shown to be concentration dependent. The average particle size of the microspheres was 24.5 \pm 2.62 μ m at a concentration of 1% CH and 48.5 \pm 2.34 μ m at a concentration of 3% CH. The resulting particle is small enough to be safely deposited in the nasal cavity, rather than traveling to the lungs. [19-21] According with these results, when the ratio of LT to CH is increased from 1:1 to 1:4, the zeta potential of the produced microspheres increases from 28.3 to 51.4 mV, respectively. [27] Particle size distribution was also found to be rather limited, with a PDI as low as 0.473. Previous research has shown that a diameter of >5-10 μ m is ideal for nasal microspheres. Particles less than 5 μ m are able to reach the lungs unimpeded, but particles 5 μ m and bigger may deposit on the nasal mucus membrane, with the larger particles settling at the front of the nose. [33,34] In our research, we found that the microsphere's particle size scaled directly with the quantity of CH utilized, and that at high concentrations, clusters of microspheres formed. This might be due of the aggregation of smaller particles into bigger ones, which in turn increases the viscosity of the CH solution.

Table 3: Characterization of lamotrigine loaded chitosan microspheres

Formulation code	Percent yield* (%\pmSD)	Particle size# (μm\pmSD)	Encapsulation efficiency* (%\pmSD)	Percent bioadhesion* (%\pmSD)	Swelling index* (%\pmSD)
F1	65.3 \pm 1.41	24.5 \pm 2.62	86.15 \pm 0.98	65.66 \pm 2.04	95.89 \pm 2.23
F2	86.7 \pm 1.53	25.19 \pm 1.08	84.91 \pm 1.46	68.51 \pm 1.78	98.58 \pm 2.65
F3	94.2 \pm 2.43	48.52 \pm 2.34	76.73 \pm 1.54	70.08 \pm 2.31	115.39 \pm 3.21
F4	95.8 \pm 0.85	75.12 \pm 3.66	72.18 \pm 2.32	85.75 \pm 1.83	124.23 \pm 2.99
F5	82.3 \pm 1.92	35.89 \pm 1.78	67.15 \pm 2.17	64.08 \pm 2.54	97.54 \pm 1.76
F6	80.5 \pm 1.07	30.75 \pm 0.97	63.84 \pm 1.26	60.54 \pm 1.48	98.27 \pm 2.51
F7	68.7 \pm 2.85	36.32 \pm 1.65	59.47 \pm 1.08	70.39 \pm 1.14	85.16 \pm 2.45
F8	74.8 \pm 2.09	38.6 \pm 1.40	54.29 \pm 1.38	72.18 \pm 1.29	82.77 \pm 2.28
*Values expressed as mean \pm SD, n=3, #indicates average of 100 particles \pm SD. SD: Standard deviation					

Morphology

Prepared microspheres' morphology was analyzed using SEM. [Figure 5] demonstrates that the LT-loaded CH microspheres maintained their regular form and smooth surface. The number of people offering free medicine samples was quite low. This demonstrates that LT is distributed across a dense polymeric network, the product of isotropic gelation. The absence of surface ruptures in the resultant microspheres provided further evidence of

their rapid clearance from the nasal canal and excellent penetration.

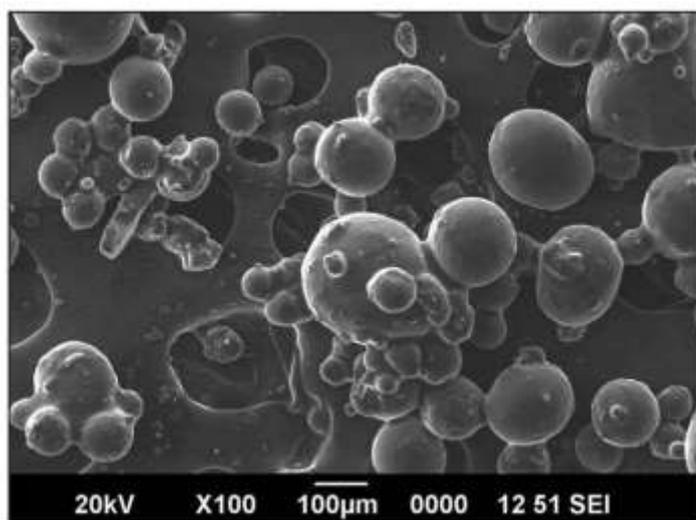


Figure 5: Scanning electron microscopy image of lamotrigine loaded chitosan microspheres

CONCLUSION

In this research, we have used the ionic gelation process to create and optimize LT-loaded CH mucoadhesive microspheres for nasal delivery. Emulsification-solvent evaporation was used to effectively entrap Lamotrigine in PLGA based nanoparticles in this study. To optimize for the required features, 32 complete factorial designs were used with varying PLGA and Poloxamer 407 concentrations. The LTG was successfully encapsulated inside the PLGA matrix and exhibited excellent amorphization using the optimized formulation. According to DLS measurements, LTG-PNPs have a nanometric size and a narrow size distribution, and their dissolution patterns demonstrate a biphasic release pattern, providing loading and maintenance dose. Goat nasal mucosa was removed for cytotoxicity testing, and the results indicated that PLGA nanoparticles were a safe delivery vehicle. Administration of LTG-PNPs intranasally resulted in sustained release, increased bioavailability, and improved brain targeting via crossing the blood-brain barrier. The discovered formulation might be given as a once-daily formulation in epilepsy, which would be useful in the treatment of epilepsy and lower the dosing frequency, dosage, dose-related adverse effects, and cost of therapy. Proof of concept from these investigations, however, has to be confirmed in higher animal models and human volunteers.

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