Frontiers in Health Informatics
ISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2024; Vol 13: Issue 8
Open Access

FORMULATION DEVELOPMENT OF LAMOTRIGINE NANOPARTICLE LOADED *IN SITU* GELS FOR NASAL DELIVERY - *IN VITRO*, PHARMACOKINETIC AND PHARMACODYNAMIC STUDY

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Cite this paper as: Mamatha Kola, R. Nagaraju (2024) Formulation Development Of Lamotrigine Nanoparticle Loaded In Situ Gels For Nasal Delivery - In Vitro, Pharmacokinetic And Pharmacodynamic Study. *Frontiers in Health Informa 3911-3923*

ABSTRACT
Lamotrigine belongs to the class of phenyl triazine and utilized for the epilepsy disorder and neuropathic pain. The aim of the study was to prepare and characterize a novel, nanoparticulate lamotrigine formulation for nasal administration. In current study, Lamotrigine nanoparticles were developed and incorporated in to *in situ* gel. Lamotrigine undergoes hepatic first pass metabolism which can be avoided via nasal administration. The Lamotrigine nanoparticles were formulated by modified ionotropic gelation method and were incorporated in to *in situ* gel. The prepared nanoparticles were evaluated for their shape, polydispersity index, particle size, zeta potential, *in-vitro* drug release pattern and encapsulation efficiency. LG4 nanoparticle formulation showed spherical shaped nanoparticles with particle size of about 82.16±0.4 nm, zeta potential of 39.4±1.44, and acceptable entrapment efficiency of 84.14±0.18, *in vitro* release of nanoparticles showed 98.31±1.38 drug release after 12 hours. *In situ* gels were prepared and the optimized gel was selected for incorporating nanoparticles. Prepared nanoparticulate *in situ* gel formulations were characterized for *in-vitro* gelation, gelation temperature, mucoadhesive strength, viscosity and drug release. The release of drug from optimized nanoparticulate *in situ* gel obeyed Korsmeyer-Peppas model. Stability study revealed that the optimized preparation was more stable at refrigerated conditions (4–8° C). Pharmacokinetic studies revealed the enhancement of bioavailability in terms of AUC and F, there was 2-fold increase in bioavailability of nanoparticle incorporated *in situ* gel in comparison with lamotrigine solution. Lamotrigine nanoparticle incorporated *in-situ* gel revealed potent anticonvulsant activity effect on the Pentylenetetrazol induced seizure model in mice.
Key words: nanoparticles, *in situ* gel, ionotropic gelation, lamotrigine

INTRODUCTION:
Epilepsy is characterized by seizure that can switch from partial seizure to generalized seizure. Due to lamotrigine's lipophilic nature, which causes it to enter and exit the brain fast, a formulation that could encapsulate the medication and allow it to remain in the brain region for a longer amount of time is required. *In situ* gel-loaded chitosan nanoparticles were formulated in the current study for nasal administration. The dose can be directly infused into the nose cavity and reach brain regions through the intra-nasal route via olfactory pathway. A quicker start of action is provided by the nasal route as the drug directly reaches brain by trigeminal

and olfactory neuronal pathway circumventing blood brain barrier.[1]However, high amount of doses cannot be given due to nasal anatomical features. The drug permeability is a problem as well, particularly for hydrophilic medications, which results in limited bioavailability in brain regions. According to reports, the use of nanocarriers improves the permeability of medications via the nasal route and offers a workable solution for the nose-to-brain pathway.[2]

Because of poor solubility and permeability, significant hepatic metabolism, short half-lives, and most importantly the existence of natural barriers like the blood-brain and blood-cerebrospinal fluid barriers, the majority of CNS medications are ineffective.[3]. The aforementioned obstacles can be addressed by the use of nanocarriers or by delivering medications through pathways that avoid the blood-brain barrier. There are four methods that avoid the blood-brain barrier: intracerebral, intracerebroventricular, intravenous, and intranasal. Because of their high level of invasiveness, the first two stated routes are not as popular.[4.] Since it offers a quick start of action and maximum drug absorption, the intravenous route is one of the most commonly used ones. The non-invasive intranasal route is utilized for selective brain targeting in order to get over the blood-brain barrier.[5]

The current study aims to prepare lamotrigine nanoparticle incorporated *in situ* gel for management of epilepsy for delivery via nasal route. The drug has poor aqueous solubility and high protein binding showed less penetration through BBB after giving through oral route. IN route provides noninvasive delivery as compared to parenteral route in addition to avoiding systemic exposure. Chitosan nanoparticles also improve the therapeutic uptake in brain by improving nasal residence time.[6]

MATERIALS AND METHODS

Lamotrigine was received from Aavyan Laboratories (Hyderabad). Sodium tripolyphosphate and chitosan were bought from Hi Q Pharma in Hyderabad. Analytical grade reagents and chemicals were used for all other purposes.

Studies on the compatibility of drugs with polymers: The drug's compatibility with the excipients was ascertained by FTIR investigations. KBr pellets procedure was used to obtain the infrared spectra of lamotrigine and its optimized formulation using a Perkin-Elmer Fourier transform infrared spectrophotometer.

Preparation of lamotrigine Nanoparticles: Based on the ionotropic gelation of chitosan with tripolyphosphate (TPP) anions, chitosan nanoparticles (CS NP) were prepared (calvo-etal). Chitosan was dissolved in acetic acid at different concentrations, and the pH was then brought to 4.7 using 5 N NaOH.[7,8] Lamotrigine was precisely weighed and added to the chitosan solution. After adding TPP aqueous solution (0.25%w/v, 5ml) dropwise to chitosan solution during magnetic stirring at 800 rpm, nanoparticles were formed. The mixture was then processed in a high-speed probe sonicator. (Table 1). The resulting nanosuspensions underwent a 30-minute, 16,000 rpm centrifugation at 4°C. After being lyophilized, the nanoparticles were preserved for later research.[9, 10, 11]

Table 1: Formulation of lamotrigine nanoparticles

Ingredients	LG1	LG2	LG3	LG4	LG5	LG6	LG7	LG8
lamotrigine (mg)	25	25	25	**25**	25	25	25	25
Chitosan:Tpp	1:1	2:1	3:1	**4:1**	5:1	6:1	7:1	8:1
0.25%w/v Tpp(ml)	5	5	5	**5**	5	5	5	5

Evaluation of Lamotrigine Nanoparticles:

Physico-chemical characterization of Lamotrigine nanoparticles

Lamotrigine (LG) nanoparticles were subjected for various parameters like morphology, particle size, entrapment efficiency and *in vitro* drug release studies[12,13,14,15]

Particle size analysis: Zeta-potential and particle size measurement was analyzed by Horiba SZ 100, dynamic light Scattering by laser Doppler electrophoresis.

Surface morphology: Using Zeiss scanning electron microscopy, the surface shape and particle size of the LG nanoparticles were investigated.

Drug Entrapment Efficiency: The drug-loaded nanoparticles were centrifuged for 30 minutes at a high speed of 6000 rpm. The supernatant was then diluted and tested using a UV spectrophotometer to assess absorbance at 310 nm for the presence of unbound drug. The following formula was used to determine the Drug Entrapment Efficiency (DEE).

$$\text{DEE } \% = \frac{\text{Amount of drug actually present}}{\text{Theoretical drug load expected}} \times 100$$

Percentage yield: Initial and final weights of the raw ingredients and nanoparticles can be calculated to find the % yield.

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$$\text{Percentage yield } = \frac{\text{Practical yield(weight of the nanoparticle)}}{\text{Theoretical yield(wt of raw materials)}} \times 100$$

In vitro release study: The dialysis bag diffusion technique was used to conduct the *in vitro* release. A certain amount of nanoparticle dispersion was added to a dialysis bag (molecular weight cutoff: 12,000–14,000 Da). The filled dialysis bag was submerged in 100 mL of phosphate buffer pH 6.6, which was set at 100 rpm at 37°C±0.5°C in a beaker. At time intervals of 1.0, 2.0, 4.0, 6.0 and 8.0, 12.0, 14.0, 16.0, 20.0 and 24 hours, aliquots were taken out. A UV spectrophotometer was used to measure the drug concentration at 310 nm. Each experiment was conducted thrice, and the outcomes were reported as mean values ± SD.

Zeta potential: The zeta potential was determined using Laser doppler microelectrophoresis.

Preparation of nasal *in-situ* gel (NIG)using Ion sensitive polymers (sodium alginate):[16]

Ion sensitive gel was prepared by dissolving Benzalkonium chloride and Sodium metabisulphite in distilled water. Sodium alginate was added and stirred for dispersing. Hpmc and Carbopol 934P were added to the above dispersion as depicted in table 2 and allowed to hydrate overnight dispersion obtained above was stirred at 1000 rpm for 10 mins with

mechanical stirrer.[17]

NIG bases using ionotropic polymers (sodium alginate) were prepared and evaluated for clarity, gelling time and gelation temperature. Many gel formulations were prepared. (as discussed in our previous publication).[18]The following table shows optimized formulation for plain ion sensitive *in situ* gels.

Table 2: Composition of Optimized NIG formulation

Formulation Code	Carbopol 934p (gms)	Sodium Alginate (gms)	Hpmc (gms)
S5	**0.045**	**0.075**	**0.13**

Preparation of lamotrigine nanoparticulate *in situ* gel (NLIG):
NLIG was formulated by cold technique. Optimized lamotrigine nanoparticles (LG4) equivalent to 25 mg of LG was dispersed in to optimized formulation of sodium alginate (SA)*in situ gel* (S5) as indicated in table 3. The solution prepared was allowed to hydrate overnight at 4ºc to lead a homogeneous gel. pH of the NLIG was adjusted to 5.5 and characterized for various tests like visual appearance, pH, clarity and viscosity.[19,20]

Table 3: Composition of Optimized NLIG formulation

Formulation Code	chitosan:tpp	Carbopol 934p (gms)	Sodiumalginate (gms)	Hpmc(gms)
LG4S5	4:1	0.045	0.075	0.13

Evaluation studies of lamotrigine NLIG[21,22]
Clarity: The gel's clarity was assessed visually in good light, with the gel being viewed against a black and white background while swirling. Turbidity formation and the presence of any undesirable particles in the solution were monitored in the gel.
Gelling capacity: A drop of gel was added to a beaker filled with calcium chloride solution, and the gelling time was visually evaluated to ascertain developed formulation's gelling ability

Determination of p^H : Using a digital pH meter, the gel pH was measured (Elico LI 613)
Measurement of gelation temperature: The Miller & Donovan approach provided a description of the gelation temperature. Two millilitres of *in situ* gel was transferred to a test tube and submerged in a water bath. The water bath's temperature was then gradually raised. After allowing gel to acclimatise for five minutes at a time at each setting, the formulation was checked for gelation. The temperature at which the meniscus stops moving after tilting to 90 degrees is referred to as the gelation temperature.
Measurement of viscosity: The rheological characteristics of *in situ* gel compositions were assessed using a Brookfield LVDV-E Viscometer. In order to study the rheological qualities, the spindle rotational speed was increased from 0.3 to 100 rpm. The gel was kept within the sample holder, and the viscosity was measured after the spindle was lowered perpendicularly into the gel and rotated at different speeds.
Mucoadhesive strength: A tiny piece of goat nasal mucosa was cut, tied or fixed on two glass vials using thread or rubber bands, and the mixture was kept at 37°C ±2°C for ten minutes. Next, 50 mg of gel was added to the first vial, which was positioned beneath the height-adjustable balance. Meanwhile, the second vial was attached to the underside of the same balance in an inverted position. Once this height was reached, both vials were adjusted and came into close proximity for five minutes to guarantee that the nasal mucosal tissue and the

in-situ gel formulation came into contact. After then, weight was added to the unbalanced side until the vials seperate; this was reported as strength or stress in dyne/cm².

Determination of gel strength: 50 g of the gel formulation was taken in a 100 ml graduated cylinder and gelled at 37°C using a thermostat. A 35 g piston was positioned at the top of the gelled solution and allowed to pierce the gel five centimetres deep. It was measured how long it took a weight to sink 5 cm.

Spreadability: A gel sample was positioned excessively between two glass slides and pressed for five minutes with a 1000g weight to create a consistent thickness. Weight (50 g) was added to the pan, and spreadability was estimated by measuring the time the upper glass slide moved over the lower plate**.**

***In vitro* diffusion studies:** Phosphate buffer pH 6.6 was used in the Franz diffusion cell in order to conduct the *in vitro* release analysis of the NLIG. A predetermined volume of the NLIG was applied to the diffusion membrane in the direction of the donor compartment, which is equal to 25 mg of lamotrigine. Release media was replaced with an equivalent volume at intervals of rotation. Filtered samples were used to measure the amount of medication released. UV- spectrophotometer (UV-2600 SCHIMADZU) was used to measure the drug concentration spectrophotometrically at 310 nm. Results were presented as mean values ± SD for each experiment, which was conducted three times**.**

Stability studies:[23] The optimized NLIG was examined for clarity, drug content, gel capacity, and *in vitro* release study at room temperature (25°C±2°C) with 60% relative humidity (RH±5%) and under refrigeration (4°C±2°C).

Pharmacokinetic study:[24]
Male rabbits were randomly divided into two groups. One of the groups received intra nasal formulation whereas the other group was treated with oral suspension. Blood samples were drawn from the right marginal ear vein into heparinized micro-centrifuge tubes at prescribed intervals following LG dosage (0.25, 0.5, 1, 2, 4, 8, 12 and 24 hours). The LG was estimated using the HPLC equipment with a UV detector set to 310 nm in wavelength.

Pharmacodynamic studies[25]
Pentylenetetrazol model: Male Swiss albino mice weighing 25 to 35 g were used for the test. The mice were placed into three groups, each with six animals. PTZ was administered intraperitoneally (i.p.) to all groups at a dose of 100 mg/kg weight, which was sufficient to cause seizures in every animal group. The first group functioned as the typical control. One hour prior to PTZ administration, the animals in the second and third groups were given a therapeutic dose of an optimized LG NLIG formulation (intranasally) and a lamotrigine solution (orally) respectively, equivalent to 0.0325mg/kg at early in the morning after 4 hrs of fasting. The animals were kept under observation immediately after PTZ injection for a certain period of time around 30 min. All the animals were observed for seizures.

RESULTS AND DISCUSSION

Drug polymer compatibility studies
Using an IR spectrum, the drug-polymer interaction was evaluated. There was no discernible shift in the peaks of the FTIR spectra of the optimized NLIG (Figures 1 and 2) compared to the pure drug, suggesting that the polymers utilized in the formulation had no effect on the drug.

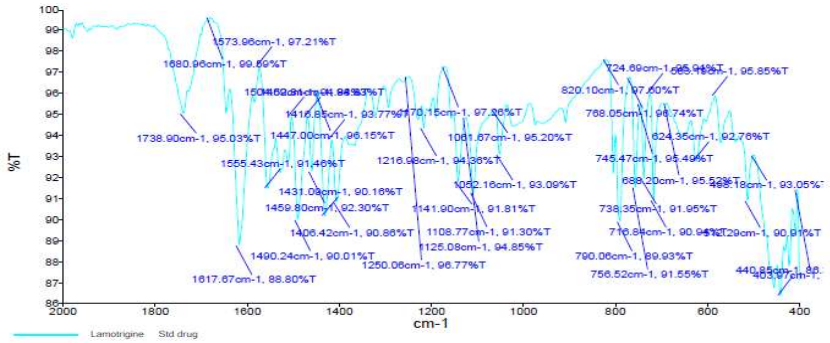

Figure1: FTIR - lamotrigine drug

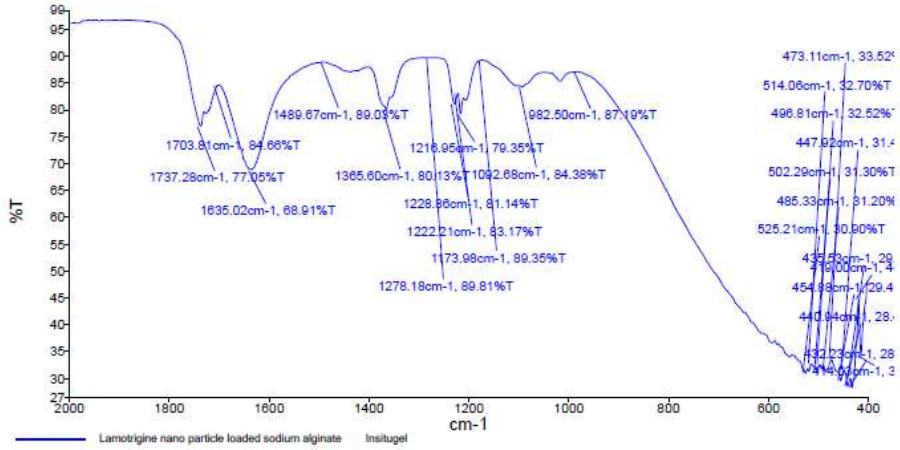

Figure 2: FTIR of lamotrigine nanoparticle loaded *in situ* gel

Optimization of LG loaded nanoparticles:

Different formulations of LG-loaded nanoparticles (LG1–LG8) were developed using the ionic gelation process. Particle size, zeta potential, encapsulation efficiency and drug release were assessed for each formulation.

Results showed that formulation LG4(CHT: TPP ratio- 4:1) was optimized formulation with particle size of about 82.16 ± 0.4 nm, PDI (0.42) and acceptable entrapment efficiency (84.14±0.18%). Different CHT:TPP ratios (1:1, 2:1, 3:1 ,4:1, 5:1, 6:1, 7:1, 8:1) were investigated LG1-LG8, CHT:TPP optimum ratio was found to be 4:1(LG4)

During optimization of CHT:TPP ratio, it was noted that a high concentration of Chitosan was unfavorable, as formulations LG6, LG7 and LG8 showed greater particle size, possibly as a result of larger chitosan conc and resulted in aggregation of NPs leading to increase in particle size.

LG4 formulation released maximum amount 98.31±1.38**%**of for 12 hours in 6.6 pH buffer(Figure 3). Drug release from LG4 formulation followed Kors Meyer peppas model. LG4 was selected as the best nanoparticle formulation to incorporate in to *in situ* gel. SEM images of LG4 showed that nanoparticles formed were spherical in shape (Figure 4), lesser PDI of 0.42 indicates nanoparticles have uniform size distribution, zeta potential of 34.5 mv (Figure 4) indicates stability of nanoparticles.

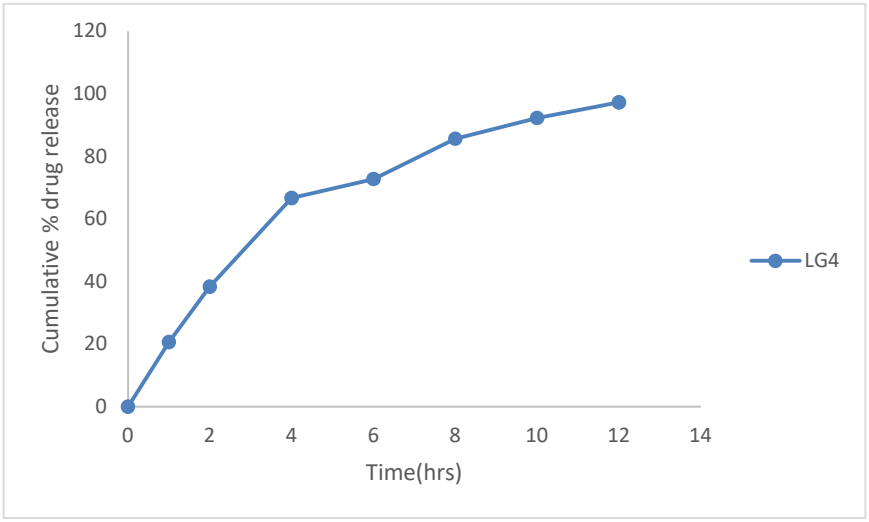

Figure 3: *In vitro* drug release of optimized LG4 nanoparticle formulation

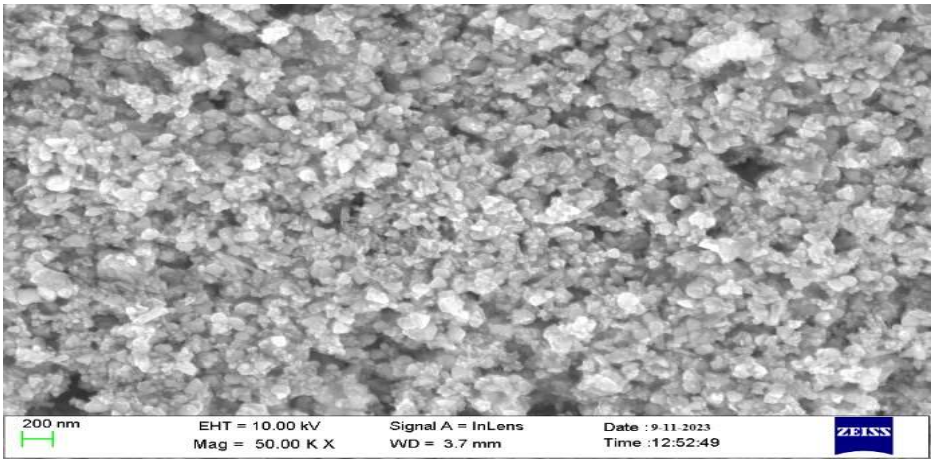

Figure 4: SEM image of optimized lamotrigine nanoparticles

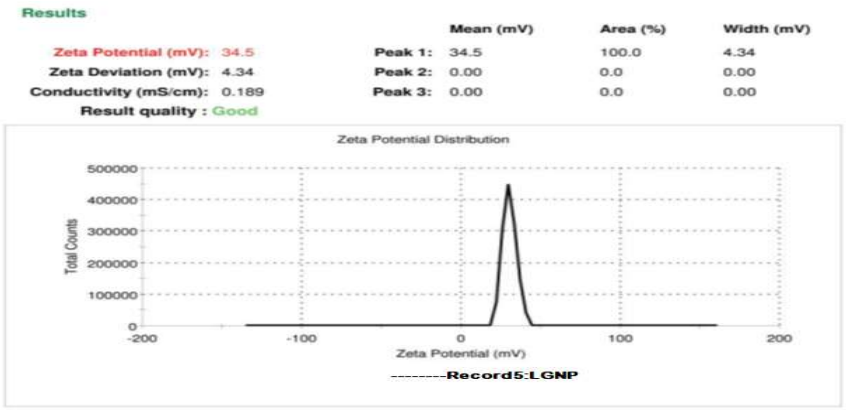

Figure 5: Zetapotential of optimized lamotrigine nanoparticles

Characterization of optimized LG nanoparticle loaded *in situ* gel

Nanoparticulate loaded *in situ* gel showed good clarity and appearance, pH of the nanoparticle laden *in situ* gel was within the nasal range. LG NLIG exhibited good gelling ability, the gelation temperature was found to be near to body temperature 37ºc, incorporation of lamotrigine nanoparticles did not show significant change in gelation temperature. LG4S5 exhibited gelation time of 35sec and this duration was optimum for nasal delivery. (Table4)

Table:4 Characterization of optimized LG nanoparticle loaded *in situ* gel

Formulation code	**Clarity**	**pH**	**Gelling capacity**	**Gelation temperature**	**Gelation time**	**Mucoadhesive strength (dynes/cm^2)**	**Viscosity Cps**	**Gel Strength (seconds)**	**Spreadability (gm.cm/sec)**
LG4S5 (nanoparticles in sodium alginate gel)	Clear	6.2 ±0.15	+++	37±0.2	35±0.15 sec	1789±0.17	156.6	144±0.98	24.2±0.7

Clarity:

+++: Very Clear

++: Clear

+: Turbid

Gelation:

-: No Gelation

+: gel formation occurs in few mins and remains for hours

++: gel formation immediately occurred and remains for hours

+++: gel formation occurs immediately and remains for longer periods

++++: stiff gel formation

In vitro drug diffusion: Lamotrigine NLIG and lamotrigine solution *in vitro* drug diffusion investigations were carried out employing Franz diffusion cell in PBS pH 6. 6 over a 24-hour period. It was observed that the drug diffusion from LG solution was much higher in short period of time compared to optimized formulations, which may be due to absence of rate limiting barrier or formulation aspects to control the release. (Figure 7.)

In vitro drug diffusion of lamotrigine solution was completed within 7 h, whereas in LG4S5 drug release sustained up to 24 h and was found to be 99.23±0.75%.

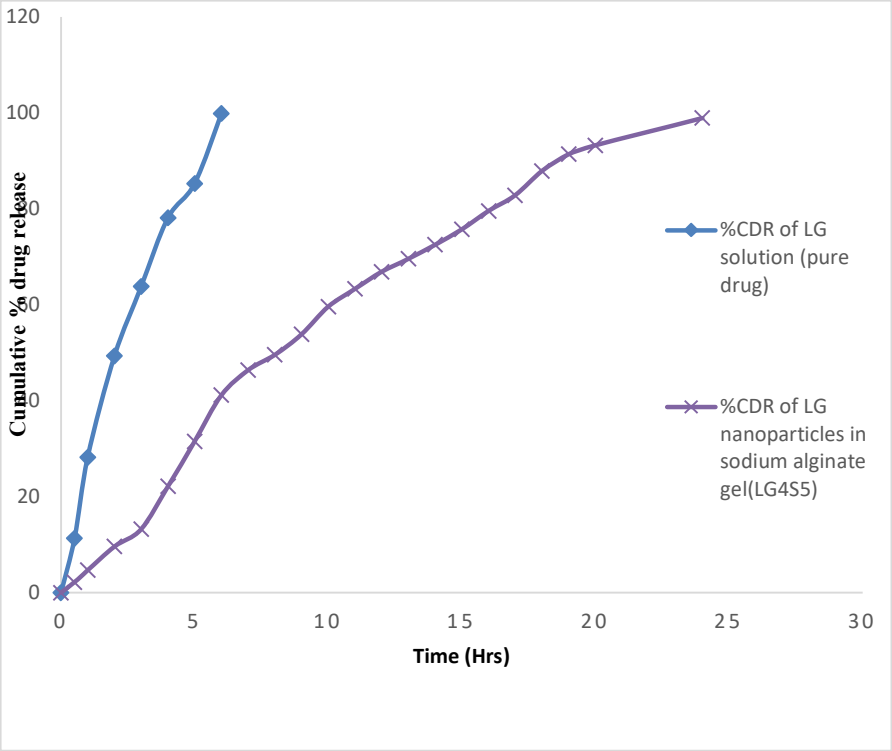

Figure 7: *In vitro* drug diffusion of Lamotrigine nanoparticle loaded *in situ* gel and lamotrigine solution

Kinetic analysis of lamotrigine release from NLIG indicates that the drug release followed Korsmeyer-Peppas (which is graphically represented between log cumulative % drug releases versus log time) with R^2 value found to be 0.9723. Percentage release of drug from gel-based formulation was comparatively slower than LG solution.

Stability studies: Stability study data indicated the influence of temperature on the stability of

NLG. There were no significant changes in the physical, chemical parameter and % drug content after storage. Overall, the NLIG formulations stored in refrigerator at 4°C was significantly more stable than the formulations stored at the room temperature. Hence it may be concluded that the prepared formulations are stable**.**

Pharmacokinetic studies: The area under the curve (AUC) for optimized NLIG and pure lamotrigine suspension (control-LG) was 449.15±16.81µg/ml.hrs. and 230.90±13.79 (µg/ml.hrs.), respectively. When NLIG was compared to control, the amount of absorption shown by AUC was considerably greater.

Gel formulation resulted in a two-fold increase in bioavailability, as measured by AUC and F ($p<0.001$) (Table 5). The prevention of pre-systemic metabolism, which is the cause of the low bioavailability of the LG, may account for the notable increase in bioavailability observed with nano loaded gel in comparison to the control. (Figure 8)

Table 5: Pharmacokinetic parameters of LG after intranasal & intravenous administration

S.No.	Pharmacokinetic parameter (mean ± SD)	Control (LG) (AM ± SD)	LG4S5 (AM ± SD)
1.	C_{max} (µg/ml)	25.36±0.46	19.65±0.4
2.	T_{max} (hrs)	2.00	4.00
3.	AUC (0-∞)	230.90±13.79	449.15±16.81

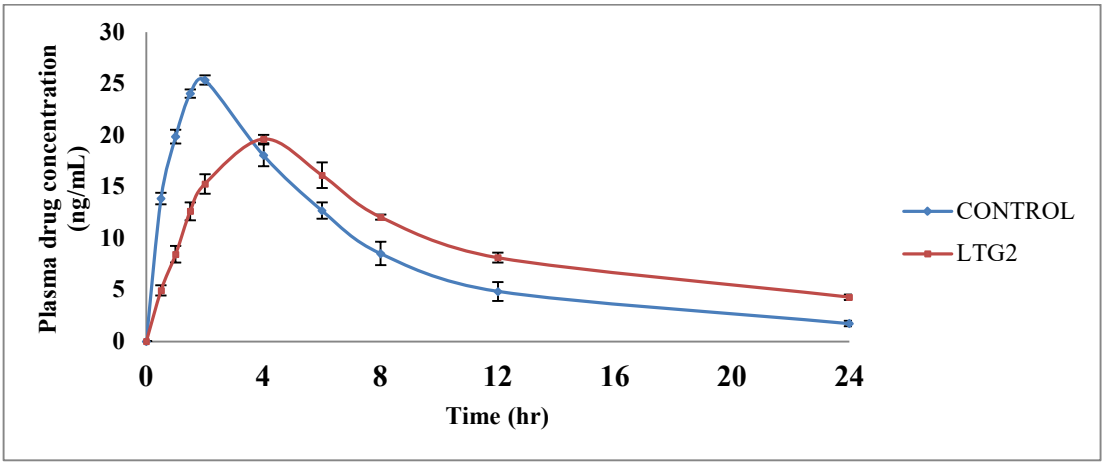

Figure 8: *in vivo* drug release of Lamotrigine solution and lamotrigine NLIG(LTG2)

Pharmacodynamic study: Lamotrigine had potent anticonvulsant effect on the PTZ experimental model. The results are depicted in the table 6. All the six animals tested in the control group at the doses of PTZ (100 mg/kg) showed latency period of around 63.3±1.63 seconds and duration of convulsions of 153.5±1.04sec.However, at the same dose of lamotrigine, intranasal delivery of Optimized-LG-NPs significantly increased the latency periods and reduced the seizure duration activity to 11 ± 1.09 sec. Administration of LG-nanoparticle loaded sodium alginate *in situ* gel intranasally prevented PTZ-induced seizures in Group 2 as compared to controls groups (Group 1) (table 6)The optimized gel was effective in the treatment of epilepsy owing to retention of *in situ* gel loaded with LG- NP in the brain blood capillaries.

Table 6: Antiepileptic effect of lamotrigine formulations

Group	Treatment	Served as	Route of Delivery	latency in sec	duration of convulsions in sec
I	PTZ+ Normal saline intranasally.	Normal/Control	Intra peritoneal+ Intranasal saline	63.3±1.63	153.5±1.05
II	PTZ+Lamotrigine NLIG intranasally	Test	Intra peritoneal+ Intranasal	706.17±4.5	11±1.09
III	PTZ + Lamotrigine solution Intranasal Route	Standard	Intra peritoneal+intranasal	647.5±4.04	96.8±4.3

Conclusion: In the view of resulted findings, it can be concluded that lamotrigine nanoparticle loaded sodium alginate gel was successfully prepared. Lamotrigine NLIG showed acceptable *in-vitro* evaluation parameters. *In-vivo* pharmacokinetic study proved that it showed better enhancement in absorption and bioavailability than pure drug. Pharmacodynamic study showed significant anti covulsant activity in test group when compared to standard and control group of mice. Thus, it can be concluded that lamotrigine nanoparticle loaded sodium alginate gel was successfully prepared and evaluated.

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