Latin American Journal of Pharmacy
(formerly *Acta Farmacéutica Bonaerense*)

Lat. Am. J. Pharm. **42** (2): (2023)

In vivo Pharmacokinetic studies of Antiepileptic drug-loaded polymeric nanoparticles

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Abstract:
Aim: The present study was aimed to prepare polymeric nanoparticles with an anti-epileptic drug, Lomotrigine and to enhance the bioavailability of lamotrigine by controlling the rate of drug release by polymeric nanoparticle. Method: The formulations were optimized by using Expert software design. The formulation variables which could affect the preparation were identified and optimized to get the nanoparticles with average particle size and highest drug entrapment efficacy. Formulation variables like concentration of polymer, concentration of drug and stabilizer concentration were studied against the response variables particle size and drug entrapment efficacy. Based on the optimization results, the selected formulations were subjected for pharmacokinetic studies. The various parameters such as Cmax, Tmax, Half-life was done by means of KINETICA application. The statistical analysis of the studies shown that the drug loaded sodium alginate chitosan nanoparticles possessed considerable improved drug amount on brain compared with that of drug. Results & Conclusion: The results showed, formulation shows a significant increase in volume of distribution and average residency and reduction in the plasma clearance rate. This is due to controlled release of Lamotrigine from Chitosan alginate Nanoparticles.

Keywords: Epilepsy, Polymeric Nanoparticles, Sodium alginate, Chitosan, Pharmacokinetic evaluation.

Introduction

A polymer nanotechnology approach to drug distribution has the potential to overcome problems in drug delivery, such as low solubility and permeability [1]. Innovations in nanotechnology have made it possible to create polymeric nanoparticle formulations which can change pharmaceuticals' pharmacological, biopharmaceutical, and pharmacokinetic properties [2]. At least 10–100 nanometers are the dimensions of polymeric nanoparticles (PNs). NPs are one of the most commonly used nanomaterials in nanomedicine since they are able to deliver a drug to a specific region of an organ at a lower dose, thereby increasing drug bioavailability [3-5].

In order to address drug delivery difficulties such as low solubility, low permeability, and low bioavailability, polymer nanotechnology is an appropriate drug delivery system [6]. Several pharmaceutical substances have been modified and improved through the use of nanoparticles [7]. Nanocapsules and nanospheres are two types of nanoparticles, which differ in their morphology. Many diseases can be treated with polymeric nanoparticles, including cholesteremia, using them to deliver medications [8].

A transient occurrence of symptoms and abnormal neuronal activity occurs during an epileptic seizure. One of the most common neurological disorders, epilepsy affects about 1% of the world's population. In terms of prevalence, epilepsy ranks second only to stroke. Drugs for the treatment of epilepsy have been developed in large numbers and more are in the process of being developed. While many epilepsy patients experience significant side effects, they are not able to control their seizures with the current antiepileptic drugs.

Epilepsy drugs that are available for treatment control seizures only in half of patients and show a significant decrease in seizures in 75%. There are a number of antiepileptic drugs that are commonly used against different types of seizures, such as phenytoin, carbamazepine, and topiramate [9]. A pharmacy researcher's interest is still in finding more selective, less toxic, and less side-effect-causing antiepileptic drugs. Because of the protective mechanisms of the CNS, the treatment of epilepsy on a systemic level is a challenge.

The application of nanotechnology, which involves engineered materials on a scale between 1 and 100 nm, is a promising method of delivering drugs across the BBB. Nanoparticles and nanocapsules are designed using the method of preparation. Materials such as natural or artificial polymers are used to prepare polymer nanoparticles [10]. A surface modification of polymeric nanocarriers was found to be a useful strategy for targeting drugs to the brain. Surface modification can be accomplished in two ways. In the first case, hydrophilic polymers or surfactants may be used to coat the surface, while in the second case, hydrophilic copolymers may be used as a biodegradable coating [11].

In order to act, antiepileptic drugs directly target specific ion channels, neurotransmitters, or receptors in the brain. In addition to treating epilepsy and stabilizing mood in bipolar disorder, lamotrigine is also sold under the brand name Lamictal. Certain types of epilepsy are controlled by lomotrigine. In a study of Lamotrigine, 98% of its bioavailability was found to be accounted for by protein binding, while 55% was accounted for by hepatic metabolism. It has a longer elimination half-life of about 29 hours and a higher excretion rate of about 65% in urine and 2% in feces. The longer half-life and clearance of Lomotrigine can sometimes lead to unwanted side-effects, leading to patient discontinuation and poor adherence. Drug tolerance is reduced due to certain side-effects associated with the central nervous system [12,13]. In the present study, polymeric nanoparticles with the epilepsy drug lamotrigine were prepared in order to improve the bioavailability of lamotrigine by controlling the rate of drug release by polymeric nanoparticles.

Materials And Methods

The drug Lamotrigine was received from Swarnoop Chemicals Pvt. Ltd. in India. Polymer chitosan was received from India sea foods, sodium alginate was purchased from sigma Aldrich and Pluronic F-68 were procured from S.D fine chemical.

FT-IR

On a Spectral Analysis with the Bruler FT-IR instrument, FTIR chromatogram were scanned between 400 and 4000 cm^{-1} at a resolution of $4cm^{-1}$ using potassium bromide discs. Each active ingredient (1:1w/w) was kept at 40ºC and 75%RH for 30 days in the drug and active ingredient mixture. Lamotrigine, excipients, as well as combinations of medicine and excipients were crushed, thoroughly combined with potassium bromide in a mortar for 3-5 minutes, and compressed into discs for 5 minutes using a hydraulic press at a pressure of 5 tonnes. The content of sample in KBr in between 0.2 percent & 1 percent. Pellets are put in the direction of the light, and the spectrum were collected and analysed for any signs of interactions [14-19].

Nanoparticles Formulation

Different polymer and surfactant concentrations were used to optimize drug-loaded polymeric nanoparticles. Several parameters were considered when developing the formulation: particle size, surface morphology, potential energy, *in vitro* drug release tests, and drug entrapment efficacy. A magnetic bead is used to optimize stirring speed at different polymer and surfactant concentrations. By emulsification and solvent evaporation, nanoparticles were prepared. Acetone and dichloromethane were used to dissolve lamotrigine and polymers. Under stirring at room temperature, the organic phase was injected into water containing pluronic F-68 flow rate of 10 ml per min.

Sonication with aqueous pluronic F-68 solution was used to emulsify the solution after stirring at 25ºC for 10 minutes. Reduced pressure was used to evaporate the organic phase. During lyophilization, dry powder nanoparticles were obtained by centrifuging the aqueous colloidal mixture at 25000 rpm for 1 hour. Chitosan alginate nanoparticles containing drugs were prepared using emulsification solvent evaporation. Optimising formulations involved evaluating three dependent variables: polymer concentrations, surfactant concentrations, and sonication times against the parameter's average particle size, polydispersity index and drug release (%CDR). *In vivo* studies were done on the selected formulations [20-23].

Evaluation parameters of PNs

***In vivo* Pharmacokinetic evaluation**

All the animal studies were carried out after the protocols were approved by IAEC and as per the guidelines of CPSCEA. An open labelled parallel study design was used for the pharmacokinetic study. The various parameters such as C_{max}, T_{max}, Half-life, MRT, Clearance and Volume of distribution, AUC 0-t and AUC 0- ∞ were determined by using KINETICA software. Approximately 200–250 g albino rats were used for the studies. Rats were fed ad libitum water and fasted overnight before experimentation. Four groups of rats were randomly divided and administered as follows. First group consisted of Lamotrigine pure, second and third groups consisted of Lamotrigine NPs formulated differently, and the fourth group served as a control group.

Blood plasma concentrations of the drug were measured several times after administration. An anticoagulant was added as disodium ededate to the micro centrifuge tubes after collecting blood samples at 0, 0.25, 0.5, 1, 2, 4, 6, 8, 20 and 24 hours via cardiac puncture. Using anticoagulant and centrifuged at 2000 rpm for 30 minutes, the collected blood was mixed with the anticoagulant. A storage solution of 21ºC was used to keep the separated plasma until it was analyzed. Brains and other tissues were collected at the same time as blood was collected. By using the HPLC method, drug concentrations in blood and brain were measured after rinsing the separated tissues with saline and homogenizing them with different volumes of saline phosphate buffer at pH 7.4 [24-28].

Stability protocol

The dispersion of polymeric nanoparticles used in this study was optimized. Two batches of each formulation were tested. Each batch consisted of three test tubes containing samples. Labels were attached to each test tube with the dates 3rd, 6th, and 12th. To protect the test tubes from light deterioration, aluminium foil is carefully placed over them. The refrigerator was used to store one batch of samples at 2–6°C. In another batch, 60 percent of the time was spent at room temperature at 25°C±2°C. Each sample was assessed for particle size (nm), zeta potential, PDI, and entrapment efficiency (percentage) under both storage conditions. Consistency was examined for each formulation [29,30].

Result and discussion

FT-IR studies

Through FTIR analysis, it is possible to evaluate the implications of different functional groups of guest and host molecules by examining significant shifts in the shapes and positions of their absorbance bands. The infrared spectrum of chitosan is shown in Figure 1. An N-H and O-H stretching band as well as an intermolecular hydrogen bond band can be detected in the 3448-3382 cm^{-1} range. In C-H symmetric and asymmetric stretching, 2921 and 2877 cm^{-1} are the absorption bands. There are similar bands in the spectrum of other polysaccharides that are polysaccharide-specific. Bands with frequencies of 1662 cm^{-1} (C=O stretching of amide I) and 1379 cm^{-1} (C-N stretching of amide III) indicate residual N-acetyl groups.

An N-H bending of amide II is observed at 1550 cm-1. N-acetyl groups will have this band as their third band. A band at 1585 cm^{-1} indicates the primary amine's N-H bending. It was confirmed that the CH2 bending and CH3 symmetrical deformations were present at 1421 and 1379 cm^{-1}, respectively. A C-O-C bridge with irregular stretching causes the absorption band at 1155 cm^{-1}. C-O stretching is characterized by bands at 1074 and 1029 cm^{-1}. Spectra of chitosan samples taken by others revealed all of the bands. These figure shows how asymmetric and symmetric carboxylate anions' stretching vibrations are caused by absorption bands of 1606 cm^{-1}, 1421 cm^{-1}, and 1319 cm^{-1}, respectively. Stretching vibrations of the hydroxyl group are seen at 3234 - 3483 cm^{-1}. 2935 cm^{-1} is the frequency of C-H symmetric and asymmetric stretching vibrations. A spectrum of lamotrigine (-NH- group) is characterized by high absorption bands at 3450 cm^{-1}, 3315 cm^{-1}, 3267 cm^{-1}, 3213 cm^{-1}, all of which are representative of amines. A doublet of carbonyl stretching mounts at 1620cm^{-1} (C=O stretching) and 792cm^{-1}, suggesting aromatic rings are present. Lamotrigine has no effect on polymers or formulations.

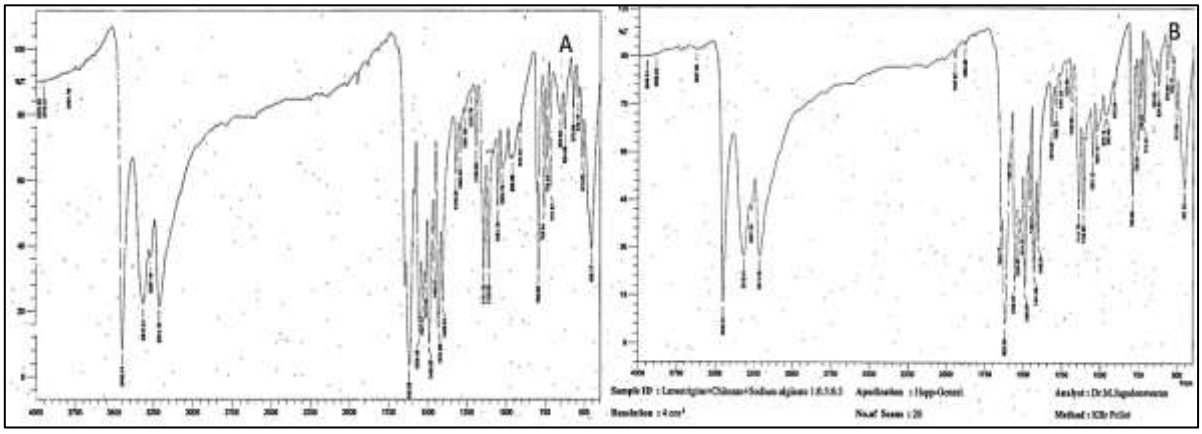


Fig. 1: FTIR spectrum (A) Pure Lamotrigine; (B) Physical mixture contains (Lamotrigine: Chitosan: Sodium alginate 1:0.5:0.5)

Table 1: Physiochemical characterization of Lamotrigine polymeric nanoparticle

** values are the measured as mean standard deviation ± SD, n=6*

Name of the formulation	Size of the particle (nm)	Zeta Potential	Polydispersity	% CRD at 24 hr	Drug Content (mg)	Entrapment Efficiency (%)
Lamotrigine nanoparticle	168.7 ± 2.46	-33.4 ± 2.32	0.328 ± 0.019	76.46 ± 2.46	6.91±0.43	89.11±4.33

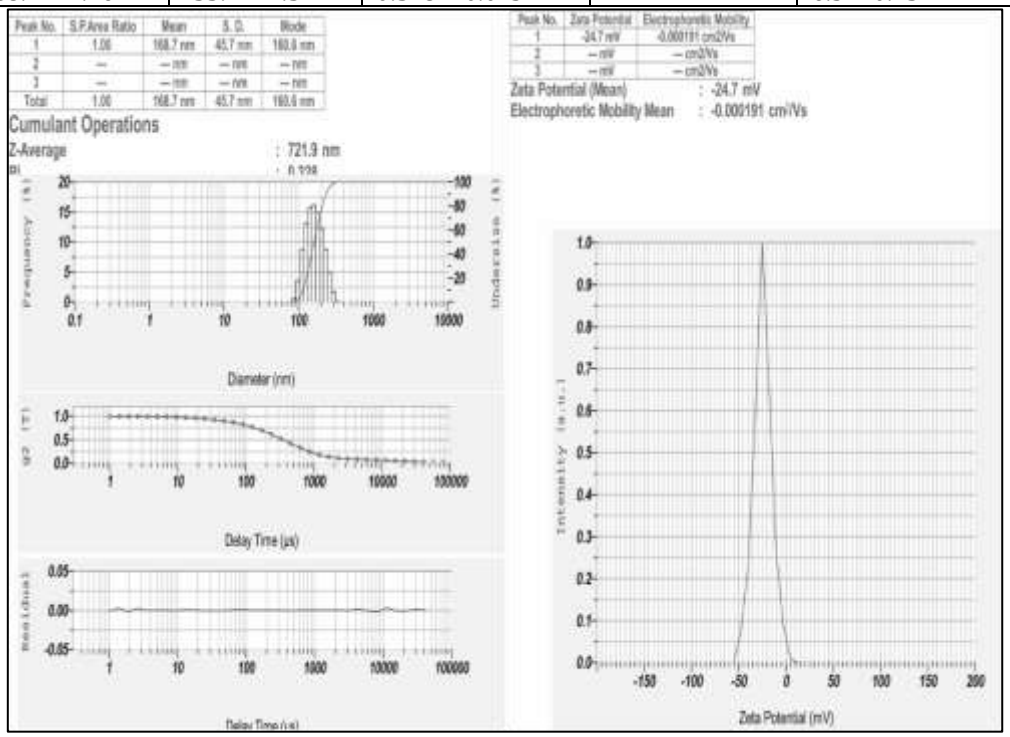


Fig. 2: Particle size, Zeta potential and Polydispersity index of Optimized Polymeric nanoparticle

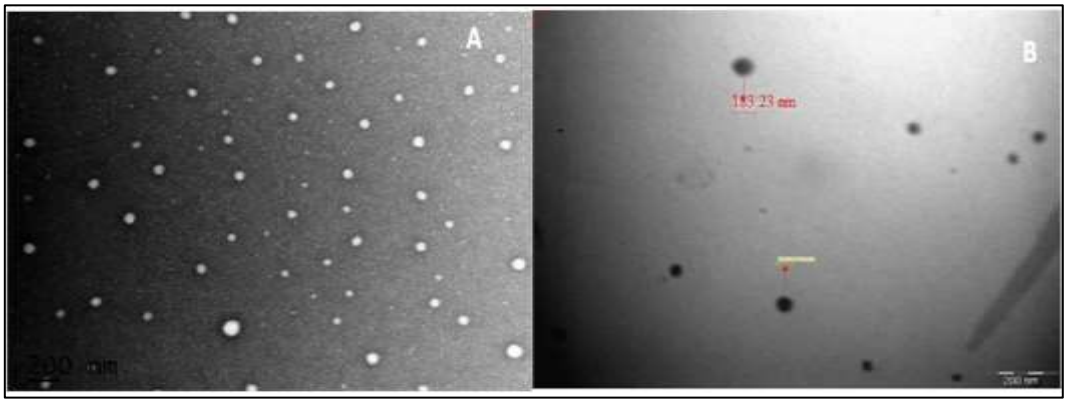


Fig. 3: SEM Morphological Studies of Optimized Polymeric nanoparticle

In this research, imaging spectroscopy (SEM) was used to analyse the morphology of the nanoparticles (Figure 3). The formation of spherical nanocomposite nanomaterials with a flat surface and no surface fracturing or pitting was revealed by SEM photos.

Physiochemical characterization of nanoparticles

Optimization design was used to characterize the lamotrigine nanoparticles in the optimized formulations. A table showing the average size of the particle, PDI, surface potential, concentration of drug, entrapment efficacy, as well as *in vitro* release studies of Line loaded nanoparticles is shown in Figure 2. According to the results, the optimized formulations were subjected to SEM, *in vitro* & *in vivo* pharmacokinetic studies.

Figure 3 shows SEM analysis of Optimized Lamotrigine Polymeric nanoparticle (LP) formulation, in which the morphology of polymeric nanoparticles was observed as mono disperse particles with smooth round surfaces. As a result, polymeric nanoparticles achieve high drug loading efficiency of 89.11±4.33%. Additionally, it was confirmed from this data that drug concentrations were uniformly distributed throughout the nanoparticles. *76.46 2.46 % drug release was observed at 24 hours in vitro for Chitosan alginate Lamotrigine Nanoparticle formulations.* Replicating the Invitro drug release studies shows a good reproducibility of drug release pattern, which suggests that drug was uniformly loaded into nanoparticle batches and was released by non-fickian diffusion.

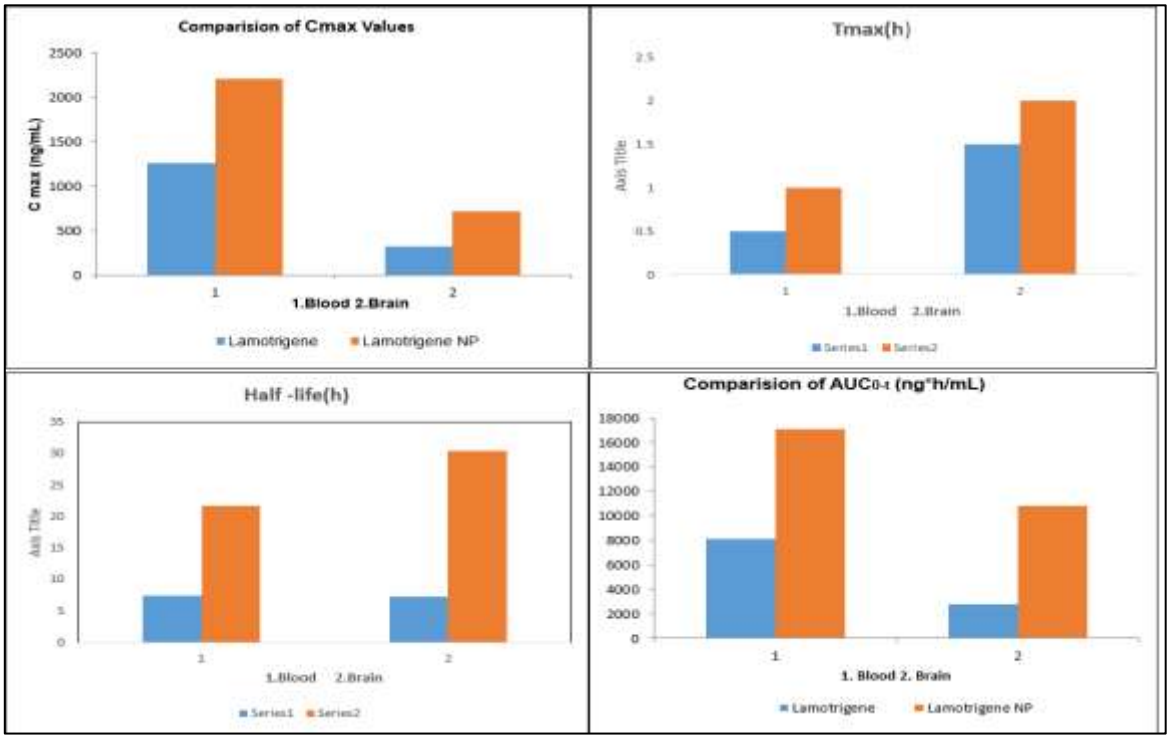
***In vivo* Pharmacokinetic evaluation**

Lamotrigine was compared with Chitosan alginate nano formulations for pharmacokinetic parameters. The Cmax of lamotrigine in blood and brain was significantly higher when administered as a nano formulation loaded with the drug than when administered as a pure drug solution, as shown in Table 2 and Figure 4.

Table 2: Pharmacokinetic parameters of Chitosan alginate nanoparticles Containing Lamotrigine

Kinetic Studies	Cmax (ng/mL)		AUC_{0-t} (ng*h/mL)		$AUC_{0-\alpha}$ (ng*h/mL)		Tmax (h)		Half-life (h)	
	Blood	Brain	Blood	Brain	Blood	Blood	Brain	Blood	Brain	Blood
Lamotrigine	1261	320	8130	2760	9760	1.754	3.324	1.87	4.38	5.34
Lamotrigine NPs	2204	721	17077	10810	68123	0.234	0.178	1.20	2.12	18.54

Fig. 4: Comparative Pharmacokinetic parameters of Chitosan alginate nanoparticles Containing Lamotrigine in blood Vs. Brain



In comparison to pure drug solution, Chitosan alginate nanoformulations containing Lamotrigine reached higher concentrations in brain. The pharmacokinetic parameters such as Tmax, AUC, MRT and t 1/2 of drugs loaded in nanoparticles were much higher than Lamotrigine (Figure 4). The drug concentration was measurable up to 24 hours after ingestion due to the slow clearance rate. Results showed significant increases in VOD and MRT of drug-loaded nanoparticles and a reduction in plasma clearance with drug-loaded nanoparticles. Chitosan alginate nanoparticles release Lamotrigine in a controlled manner.

Stability Studies

A series of long-term stability studies for Lamotrigine polymeric nanoparticles were conducted at a variety of temperatures (four degrees Celsius and twenty degrees Celsius and sixty degrees Fahrenheit) and the results are presented in Figures 5 and 6. The parameters were assessed every three months. In stability studies, the evaluation parameters for Lamotrigine polymeric nanoparticles did not significantly change. The Lamotrigine polymeric nanoparticles were stable at varying temperatures and humidity levels.

Table 3: Long term stability studies for lamotrigine nanoparticle

Parameter	**Stability studies data of lamotrigine nanoparticle**					
LM PN	**TEMPERATURE**	**INITIAL**	**AFTER 3 MONTH**	**AFTER 6 MONTH**	**AFTER 12 MONTH**	**ACCEPTANCE CRITERIA**
PS (nm)	4˚c ± 2˚c	168.7 ± 2.46	168.8± 2.28	168.8 ±2.12	169.4± 2.24	10 to 500nm
	25˚c ± 2˚c/ 60% RH	168.7 ± 2.46	168.9. ±2.24	169.7 ±2.46	170.7 ± 2.24	
Zeta potential(mV)	4˚c ± 2˚c	-37.2 ± 2.32	-33.6 ± 2.68	-33.6 ±2.28	-35.2 ± 2.26	±30 to 60 mV
	25˚c ± 2˚c/ 60% RH	-37.2 ± 2.32	-34.6 ± 2.44	-35.6 ±2.28	-36.5 ± 2.46	
PI	4˚c ± 2˚c	0.328±0.019	0.344±0.024	0.446±0.022	0.548±0.022	<1
	25˚c ± 2˚c / 60% RH	0.328±0.019	0.332±0.024	0.534±0.024	0.740±0.022	
EE %	4˚c ± 2˚c	85.91±0.43	84.89±0.24	85.46±0.64	85.04±0.28	> 85 %
	25˚c ± 2˚c/ 60% RH	85.91±0.43	86.22±0.46	86.64±0.08	84.26±0.68	
DC	4˚c ± 2˚c	86.46±2.46	85.68±2.08	84.24 ±2.36	84.03 ± 2.64	> 85 %
	25˚c ± 2˚c/ 60% RH	86.46±2.46	84.65±2.68	83.54 ±2.68	82.12 ± 2.56	
%CDR	4˚c ± 2˚c	89.11± 4.33	88.24± 4.24	87.34± 4.98	87.22± 3.06	> 85 % to 115%
	25˚c ± 2˚c/ 60% RH	89.11± 4.33	87.52± 4.20	86.72± 3.46	85.26± 4.68	

Fig. 5: Stability Studies –LM PN Particle Size reports of at (A) Initial manufacturing time (B) 4˚± 2˚ C; (C) 25˚C ± 2˚C/ 60% RH after 12 months

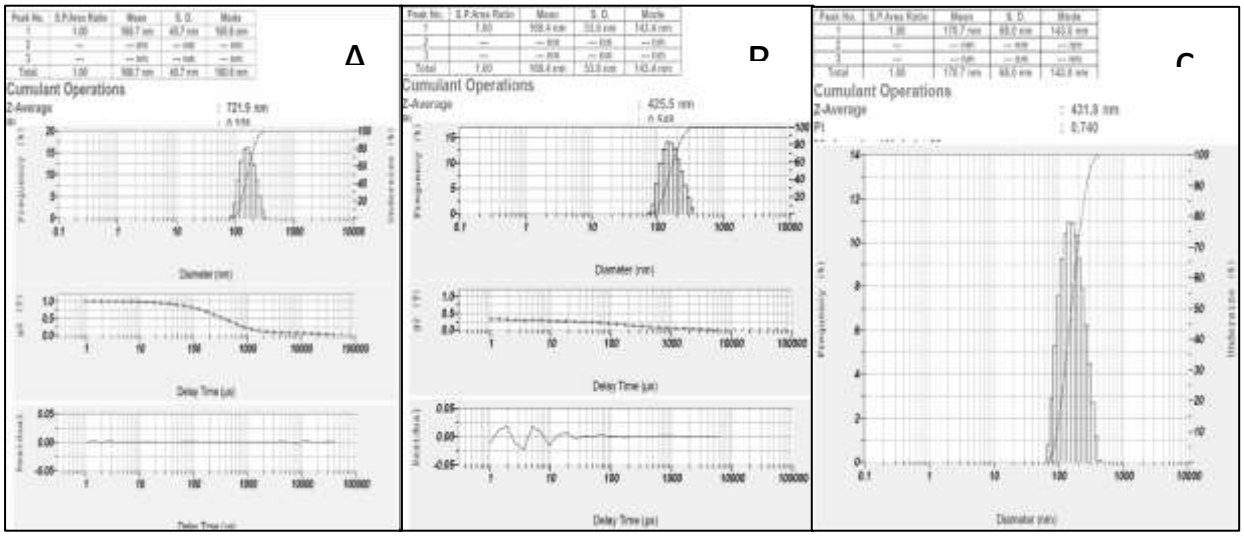
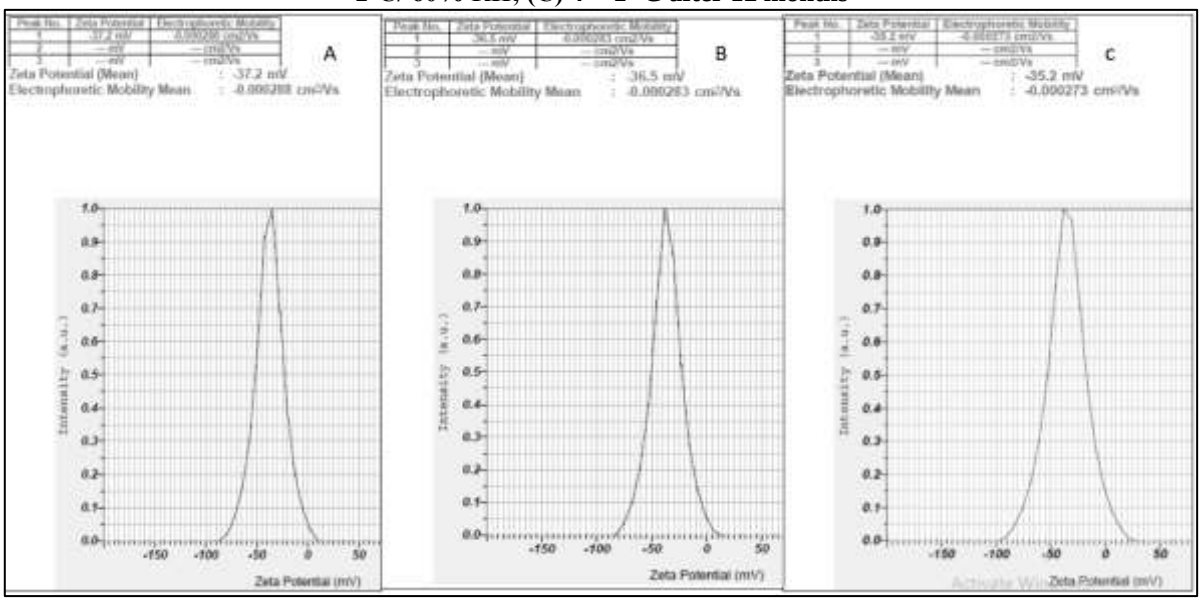


Fig. 6: Stability Studies –LM PN Zeta potential reports of at (A) Initial manufacturing time (B) 25˚C ± 2˚C/ 60% RH; (C) 4˚± 2˚ C after 12 months



Conclusion

The results showed that the formulation shows a significant increase in volume of distribution and mean residence time and reduction in the plasma clearance rate. Thus, it shows the enhancement of bioavailable dose. This is due to controlled release of Lamotrigine from Chitosan alginate Nanoparticles.

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