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A Possible Way to Improve the Therapeutic Effectiveness of Levetiracetam Via Nose to Brain.

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ABSTRACT

The nasal route of administration is an ideal alternative over parentrals for administering drug intended for systemic effect, in view of rich vascularity of the nasal membranes. Numerous potential drugs for treatment of neurological diseases are not effectively reaches to the brain in adequate concentrations to be therapeutic because of the blood brain barrier. The intention of the project was to develop formulations of Levetiracetam, a anticonvulsant, licensed for the treatment of major epileptic disorder. The delivery of drugs to the brain via the nose-to-brain way holds great assurance, on the source of preclinical study by means of drug delivery systems such as polymeric in situ gels. Formulations were evaluated for diverse physicochemical properties. *In vitro* permeation study, histopathological and stability study was also performed on selected formulations. Appearance of all formulation was found to be clear viscous, transparent and pH was in the range of 6.2 to 6.8. The gel strength was found to be affected by concentrations of gelling and bioadhesive polymers. Mucoadhesive strength of formulation was found to be excellent. In vitro release profile was found to be 98.29% at the end of 9 h and was anomalous (non Fickain) transport. The microscopic remarks specify the formulation has no significant effect on microscopic structure of mucosa.These formulations did not show any remarkable damage to nasal mucosa and retained good stability over the period of 90 days. Therefore, formulations seem to be safe with respect to nasal administration and could be utilized effectively in management of epileptic disorder.

Keywords: Levetiracetam, Nose to brain delivery, epileptic disorder, *in vitro* permeation study, stability study.

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INTRODUCTION

Nasal therapy has been renowned in ayurvedic system of Indian medicine as Nasyakarma. Traditionally nasal route has been utilized for delivery of many drugs for limited treatment of diseases like nasal congestion, allergy and infections. But now a day this route is being explored for delivery of various drugs to systemic circulation. The management of brain disorders is mostly demanding due to the incidence of a variety of formidable obstruction to deliver the drugs selectively and effectively to the brain. Blood-brain-barrier (BBB) comprises the chief obstacle to the uptake of drugs into the brain following systemic administration [1]. Direct nose to brain delivery of drug is a realistic, safe, non-invasive and suitable form of formulation approach and could be viewed as an outstanding alternative approach to conventional dosage forms. Intranasal release recommends a non-invasive and suitable method to bypass the BBB and delivery of therapeutically active agent directly to the brain.Various brain disorders such as acute seizures, status epilepsy etc organize are all complex medical problems involving numerous approaches for effectual management. Intranasal delivery of drug is raising as a low-tech, economical and non-invasive first line method for control of selected patients with these and other CNS problems. Advantages offer by intranasal drug delivery are rapid drug absorption via highly vascularized mucosa, ease of administration, non-invasive, Improved bioavailability, Improved convenience as well as compliance, Large nasal mucosal surface area for dose absorption, avoidance of the gastrointestinal tract and first-pass metabolism, rapid onset of action, lower side effects etc. but fewer are the drawbacks associated with this system such as some drugs may cause irritation to the nasal mucosa, nasal congestion due to cold or allergies may interfere with absorption of drug [2].

Levetiracetam (Keppra; UCB Pharma,Inc.,Smyrna,GA., USA) is second generation antiepileptic drugs (AED) which first received approval from the United States Food and Drug Administration (FDA) as adjunctive treatment for partial-onset seizures in adults in November of 1999. Epilepsy is a chronic condition characterized by recurrent unprovoked epileptic seizures. It affects 0.5%–1% of the population and at least 50% of patients with epilepsy have partial seizures. About 30%–70% of partial seizures are controllable with antiepileptic drugs (AEDs). The majority of these patients will need lifelong AED therapy. Strict AED compliance is often related to better tolerability and is a key factor in achieving better seizure control. An inverse relationship between the number of daily doses and compliance has been reported. Every increase in dosing frequency (from one to four doses per day) resulted in progressively worsening compliance and increased missed doses. Conceptually, the stable plasma concentration profile of extended release AED formulations is expected to minimize peak concentration–related adverse events and improve compliance and seizure control. Extended release formulations may contribute to better tolerability and improved efficacy [3,4].

In the past few years, there are increasing number of insitu forming systems have been reported in the literature for various biomedical applications, including drug delivery, cellen capsulation and tissue repair. It has long been recognized that the olfactory region in the nose is a potentially important site for entry of viruses, bacteria and foreign chemicals into the brain. In 1937, Rake used distribution tests and direct microscopical examination to show that the bacterias pneumococci and S. enteritidis entered the CNS via the olfactory mucosa and the perineural space after nasal instillation to mice. Different strains of mouse hepatitis virus [5] have also been reported to travel along the olfactory neurons into the CNS.

In situ gelling systems are the aqueous polymeric solutions that are transformed into gels due to changes in environmental conditions like temperature and pH. The insitugelsare fluids that can be introduced into the body in aminimally invasive manner prior to solidifying or gelling with in the desired tissue, organ, or body cavity. These system sare currently of interest to the formulation scientists due to their structural and functional benefits. Variety of therapeutic agents has been formulate dasinsitugelling systems for their enhanced transport a cross themucosal membranes. When the gel is formed under physiological conditions it maintains its integrity for a desired period of time, the process may provide various advantages such as reduction of post-nasal drip due to high viscosity, reduction of taste impact due to reduced swallowing, reduction of anterior leakage of the formulation, reduction of irritation by using soothing excipients and target delivery to mucosa for better absorption. Insitu forming hydrogels that exhibit mucoadhesive behaviour could be extremely useful in nasal drug delivery applications. Such gels woulds wells ignificantly when in contact with mucosa &releases the drug in steady manner while adhering tothe nasal mucosa[6].

Use of pH responsive polymers such as pluronic F127 temperature responsive polymer andion-

responsive polymer such a sxantham gum is a high molecular weight extra cellular polysaccharide, produced in commercial scale from the fermentation of gram negative bacterium Xanthomanas campestries. It is a hydrophilic polymer, which until recently had a limited use as thickening, suspending and emulsifying agent in water based systems .Xantham gum not only retards drug release, but can also provide time independent release kinetics. Apart from theses it shows desirable property such as compatibility and inertness. Release of soluble drugs from this biopolymer occurs mainly through diffusion, whereas sparingly soluble or insoluble drugs are released as a result of matrix erosion. It is also recommended for use in both acidic and alkaline media. One of the most promising poloxamer is Poloxamer 407 (Pluronic F127) because of its low toxicity, high solubility, bioadhesion characteristics, and acceptability as drug delivery vehicle. Some of the other investigated combination of poloxamer are polymer pluronic PF127 along with benzalkonium chloride which helped decreased the gelation onset temperature. This combination was prepared for the nasal delivery of Vitamin B12. In another study, poloxamer 407 was combined with a mucoadhesive polymer or polyethyleneglycol. The combination allowed manipulation of the temperature at which the conversion of sol to gel would take place as well as decreasing and increasing the in vitro release of the drug respectively [7,8]
The present study describes the development of levetiracetam in situ nasal gel in the treatment of epileptic disorders. The intention was to examine extensively the influence of developed formulations in terms of physicochemical properties viz; pH, gelation strength, gelation properties, viscosity, mucoadhesion, drug content, histopathological studies, in-vitro drug release profile & kinetics profile.

MATERIALS AND METHODS

Material

Levetiracetam was obtained as a gift sample from Zydus Cadila. Ahemdabad (GJ), India. Pharmaceutical Excipients were purchesed from chemical suppliers Sudarcshan Scientific Labortaries, Nashik and they were Pluronic F127 (Sigma-Aldrich, Pvt. Ltd. USA) Sodium Alginate (Sigma Aldrich) Xantham gum (Dow Chemical Company US). All other chemicals used of analytical grade were purchased from Jinedra Scientific Jalgaon, Maharashtra India.

Methods

Preformulation study:

Preformulation study was carried out in the following manner such as: melting point determination, Infrared Spectroscopy, and Differential Scanning Calorimetry (DSC) for the identification of drug. Drug-excipients compatibility studies such as FT-IR, DSC and X-Ray Diffractometry (XRD) were also performed. [9]

FORMULATION OF IN SITU GEL SYSTEM

In situ gels are prepared by cold method (10) A 18 % w/v Pluronic F127 was prepared in a mixture of propylene glycol/water (35:65), and then the bioadhesive agents (Sodium alginate & xantham gum) were added to it in three different concentrations of 2.5%, 3.0%, 5% w/v, and 0.1%, 0.2%, 0.3% w/v, respectively. Then 0.9% Sodium Chloride and appropriate quantities of methyl paraben was also added into the dispersion. Levetiracetam was then added to the dispersion, which was kept at 4^{o}C until a clear solution was obtained. The formulations were filled in 30 ml amber colored glass vials, capped with rubber bungs and sealed with aluminum caps. Formulations were stored in a refrigerator (4–8 °C) until use. [10]Formulation codes of the developed gels are provided in **Table I.**

Table 1: Composition of In-situ Nasal Gel Evaluation of in situ gel system

Sr No	Ingredients	Formulations Composition% w/v					
		B1	B2	B3	B4	B5	B6
1	Levetiracetam	2.0	2.0	2.0	2.0	2.0	2.0
2	Pluronic F-127	15	15	18	18	20	20
3	Sodium Alginate	2.5	-	3.5	-	5.0	-
4	Xantham Gum	-	0.1	-	0.2	-	0.3

5	Sodium Chloride	0.9	0.9	0.9	0.9	0.9	0.9
6	Methyl Parabben	0.1	0.1	0.1	0.1	0.1	0.1
7	Ultra pure water /Propylene Glycol 65:35	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

Gelation Studies

Gelation temperature was determined by pouring 5 ml formulation in a 20-ml beaker containing magnetic bar and placing it on a hot plate. A thermometer was immersed in the gel, which was heated at a rate of 2°C/min with constant stirring at 20 rpm. When the bar stopped moving due to gelation, the temperature was recorded as the gelation temperature. Similar procedure was carried out for all the formulations [11].

Drug content uniformity

100 µl of the preparation was transferred to 100 ml volumetric flasks with a micropipette and the final volume was made up with Phosphate buffer pH 6.8. Levetiracetam concentration was determined at 229 nm (UV-1700, Shimadzu,Japan). [12] Drug content was calculated taking dilution factor in to consideration by using following formula.

$$\text{amount of Levetiracetam in gel} = \frac{As \times Cr}{Ar}$$

Where, As = Absorbance of the sample solution; Cr = Concentration of the Levetiracetam in standard solution; Ar = Absorbance of standard solution

Same procedure was adopted for all prepared batches in triplicate and mean drug content and standard deviation of varies were calculated.

Viscosity Measurements

Viscosities of prepared formulations before and after gelation were measured by using Brookfield DV-E viscometer using Spindle number 3 at 100 rpm shear rate. In case of Polymers such as Pluronic, sodium alginate and xantham gum based systems the viscosity was recorded at increasing temperature in range of 20° C to 30°C and graphs were plotted.

The viscosity of gels was evaluated by treating viscosity reading using following pair of equations

$$t_i = Kat_{\alpha}$$

Where, t_i is the shear stress, Kat is the conversion factor (0.279 for spindle # 3) and α is the torque dial

$$y_i = Kny\ (n)\ Ni$$

Where, y_i is the shear rate, n is the flow index of fluid (Computed from slope of log of yi vs. log N), Kny is the Conversion factor (taken on the basis of obtained values of (n); Ni is the rotational speed. Temperature was determined as indicated by sudden rise of viscosity [13].

Determination of gel strength:

It is expressed in terms of time (s) required by a 35g piston for penetration of 5 cm distance, through the 50g gel formulation. Test was performed using 'Gel strength apparatus' modified at laboratory (Fig.6) as mentioned by [10] Gel formulation (50 g) was placed in a 100 ml measuring cylinder and gelation was induced by means of temperature. The piston (weight: 35 g) was then placed onto the gel. The gel strength was measured as the time (seconds) required moving the piston 5 cm down through the gel. In cases that took more than 5 minutes to drop the apparatus into the gel, additional weights were placed on top of the

apparatus and gel strength was described by the minimal weights that pushed the apparatus 5 cm down through the gel [11].

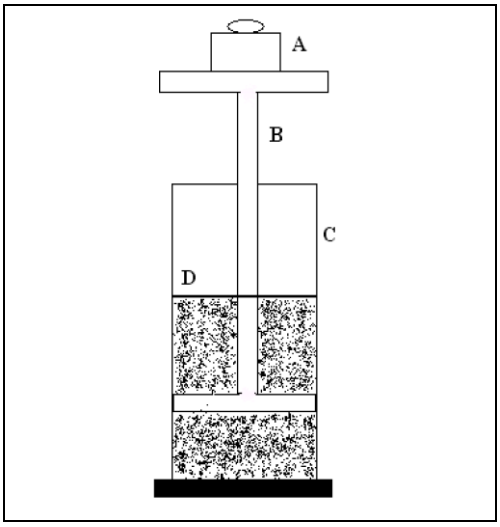


Figure 6: Gelstrengthmeasuringapparatus.

(A)Weight;(B)Shaft; (C)measuringcylinder;(D)Polymergel

Mucoadhesive strength:

Mucoadhesive potential of each formulation was determined by measuring force required to detach nasal mucous membrane from the formulation using the modified method. Freshly excised goat nasal membrane obtained from the local slaughter house was attached to the upper probe using cyanoacrylate adhesive.The upper probe was attached to pre- calibrated force displacement transducer SS12LA; (BIOPAC Systems) connected to the Student's Physiographic apparatus. The surface area of each exposed mucosal membrane was 4.2 cm^2. At room temperature, fixed amount of samples of each formulation were placed on the lower probe. The probes were equilibrated and gelation was induced by means of temperature. Probe with nasal membrane was lowered until the tissue contacted the surface of the sample. Immediately, a slight force was applied for 2 minutes to ensure intimate contact between the tissues membrane and the samples. The probe was then moved upwards at a constant speed of 0.15 mm/s.The bioadhesive force, expressed as the detachment stress in dyne/cm^2, was determined from the minimal weights that detached the tissues from the surface of each formulation using the following equation; [14]

$$\text{Detachment stress } \left(\frac{\text{Dyne}}{\text{cm}^2}\right) = \frac{\text{m} \times \text{g}}{\text{A}}$$

Where; m is the weight added to the balance in grams; g is the acceleration due to gravity taken as 980 cm/s^2 and A is Surface area of goat nasal mucosa

In vitro permeation study:

In vitro permeation study was performed using a Franz diffusion cell containing 25 ml of phosphate buffer (pH 6.8) using an excised goat nasal mucosa [10]. The goat nasal cavity was obtained from local slaughter house; within 15 min it was sacrificed. After removing the skin, the nose was stored on ice cold phosphate buffer (pH 7.4, 0.05 M). The septum was fully exposed, and nasal mucosa was carefully removed using sterile forceps and surgical scissors. The mucosal tissues were immediately immersed in Ringer's solution. The freshly excised nasal mucosa was mounted on the diffusion cell, and formulation equivalent to 750 mg levetiracetam gel was placed on it. Throughout the study, the buffer solution in the chamber was maintained at 34 ± 1°C by connecting the Franz diffusion cell with water bath. At predetermined time intervals, 1 ml of the samples was withdrawn at a time and replenished with an equal amount of phosphate buffer. The samples were diluted appropriately and filtered. Absorbances of the samples were measured

spectrophotometrically at 229 nm using UV-Vis Spectrophotometer (Shimadzu UV-VIS 1700, Japan), taking phosphate buffer (pH 6.8) as the blank. The amount of drug permeated was calculated from the calibration curve (linearity range = 2 to 14 µg/ml; $r^2 = 0.998$). The mean cumulative percentage of drug permeated was plotted against time (Fig. 3). Permeation area was 2.54 cm^2 [11]

Histopathological evaluation of mucosa:

Histopathological evaluation of tissue incubated in PBS 6.4 after collection it was compared with tissue incubated in the diffusion chamber with gel formulation (B3, B6). Tissue was fixed in 10% buffered formalin (pH 6.8), routinely processed and embedded in paraffin. Paraffin sections (7µm) were cut on glass slides and stained with haematoxylin and eosin. Sections were examined under a light microscope, to detect any damage to the tissue during ex vivo permeation study. The photographs were taken by camera [10,15].

Stability Study:

Formulations showing optimum gelation, gel strength, mucoadhesive force and drug release rate were selected for stability studies. Stability studies were carried out on B3, B6 formulation according to ICH (International Conference on Harmonization) guidelines. The stability chamber was maintained at 30±2°C, and samples were withdrawn at 0, 30, 60, 90 days interval. The physical Stability of gel was observed periodically for the occurrence of turbidity and gelation. Formulations were also evaluated at periodic time intervals of one month for the clarity, drug content; gel strength and in vitro permeation of drug [16,17].

RESULTS

The present study was aimed towards to increase the site of action and to avoid the first pass metabolism. Melting point of levetiracetam was measured; and found to be in the range of 115-120^{0}C. It was confirmed with the reported melting point of levetiracetam i.e. 118ºC. Preformulation study were carried out Drug- polymer interaction studies were performed by FTIR spectroscopy and there were no any interaction occur between their peaks all the peaks of drug was obtained in physical mixture of polymers utilized.The DSC thermogram of Levetiracetam displays an endothermic peak at 118.15ºC and In contrast, the DSC curve of Levetiracetam + Pluronic F127 of Physical mixture shows broad endothermic peaks at 58.66 ºC and. 161.54 ºC DSC curve of Levetiracetam + sodium alginate physical mixture shows less endothermic peak at 116.69 and 257.43 ºC then DSC curve of the Levetiracetam + Xantham Gum show endothermic peak at 117.43, the exothermic peak at about 226.99 ºC corresponding to the free Levetiracetam disappears. The X-ray diffraction pattern was recorded for pure Levetiracetam, Drug / PluronicF127 physical mixture and Drug/Sodium alginate, and Drug/Xantham Gum. The X-ray Diffractogram of Levetiracetam has sharp peaks shows at diffraction angle 4.8340, 12.5400, 13.4220, 17.0560, 19.5120, 21.2230, 23.2640 and 27.7710 which shows a typical crystalline pattern. The X- ray diffraction pattern of the physical mixture of Levetiracetam / PluronicF127, Levetiracetam / Sodium Alginate and Levetiracetam /Xantham Gum inclusion complex shows peaks but of low intensity indicating that some amount of Levetiracetam converts to amorphous form.

Formulations were prepared using cold method. The physical appearance of formulation was found to be clear white viscous and transparent. Total six batches were prepared; pluronic F127 was used in the various concentrations ie 15 to 20 % but according to their physical appearance, gelation and sol to gel formation phenomenon only 18% w/v was considered as an optimum concentration. Formulations B1, B3 and B5 were prepared by utilizing sodium alginate in various concentration 2.5 to 5%, but 3.5% w/v was found to be best concentration and therefore was selected for further studies. Formulations containing xanthan gum viz., B2, B4 and B6 were prepared in concentration 0.1 to 0.3% w/v. Optimum concentration 0.3% w/v was found to be best. pH of formulations was adjusted between 6.2 to 6.8; by using phospate buffer saline. The formulations were filled in 10 mL amber colored glass vials, capped with rubber bungs and sealed with aluminium caps. Formulations were stored in a refrigerator (4–8 C) until use.

Gelation studies were carried out at different temperature. In these studies the gelling capacity (speed and extent of gelation) for all formulations were determined. After easy instillation in to nasal cavity the liquid polymeric solutions should undergo rapid sol to gel transition by means of thermo sensitivity. Thus, in situ formed gel should preserve its integrity without dissolving or eroding so as to localize the drug at absorption site for extended duration. The thermosensitive polymer Pluronic F127, sodium alginate and

xantham gum is also the dual stimuli responsive in situ gelling system ideal for nasal drug delivery. The results of gelation study of the optimum concentration of Pluronic F127 was found to be B3 18% w/v. In the preliminary studies, the minimum concentration of sodium alginate containing in formulation was found to be 2.5% w/v for formulation B1, that formed gel temperature is below than 32ºC and for formulation containing xantham gum was found to be B6 (0.3%w/v), at gelation temperature is bellow than 34ºC.

Table 2: Physico-chemical Evaluation of Formulated Gel

Batch Code	Drug Content% *	Gelation*	pH *	Gel Strength*	Viscosity solution (cps)*	Mucoadhesive Force * (dyne/cm^2)
B1	99.20± 0.12	32.12±2.00	6.21±0.02	40.11±0.12	250 ± 1.15	2048.41
B2	98.23± 0.13	30.6±1.00	6.39±0.10	38.34±0.03	325 ± 1.73	2394.22
B3	99.54± 0.04	29.16 ±1.52	6.63±0.61	43.58±0.01	348 ± 2.08	2774.68
B4	98.45± 0.12	31.24 ± 2.08	6.82±0.20	43.94±0.17	373± 2.51	3378.45
B5	98.55± 0.12	29.67± 1.15	6.46±0.25	46.25±0.02	348± 1.16	4012.13
B6	98.33±0.15	33.23±1.77	6.69±0.4	61.26±0.15	490±1.24	4783.14

***Avarage of three determinations**

Table 3: Histopathological inference of Photomicrographs

Sr. No.	Histological findings	Formulations	
		PBS treated	(B3, B6)
1	Degeneration of mucosa	-	+
2	Erosion of mucosa	-	-

Where; - : No change, + : Very slight, + + : Slight

The drug content of the formulations was ranging from 98.23% to 99.45%. Drug content of Pluronic gel B3 was found to be 99.54% and for the formulation B6 was found to be 98.33 % The pH formulation containing 2% w/w levetiracetam containing different amount of polymers viz, pluronic F127, sodium alginate and xantham gum was measured. The pH value of levetiracetam gel showed in acceptable range i e 6.21 to 6.82.

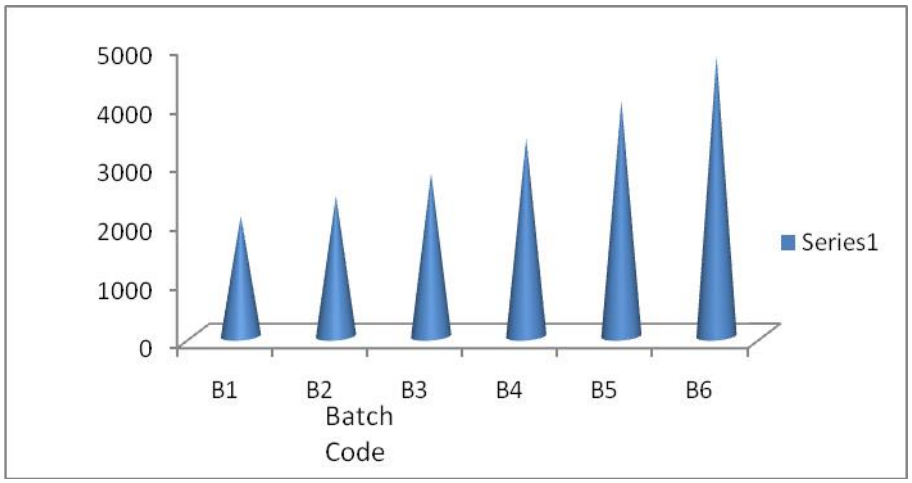
The apparent viscosity values were measured for formulations using Brookfield viscometer DV-E with spindle no. 3 at 100 rpm. The viscosity value of formulations were increased from B1 (250 ± 1.15), B3 (348± 2.08), and B5 (348±1.16) cps. was observed. It also represented that the formulation B6 possessed high values 490±1.24 cps. Viscosity of formulation B2, B4 was found to be 325 ± 1.73, 373± 2.51 cps respectively. All the formulations exhibited pseudoplastic behaviour. It was observed that all the xantham gum gels showed higher viscosity than the gels prepared from sodium alginate.

The gel strength values between 25 to 50 seconds were considered sufficient. The gel strength lessthan 25 seconds may not retain itsintegrity and mayer oder apidly whilegels having strength greater than 50 seconds are to ostiffand may cause discomfort to them ucosal surfaces. In the development of nasal in situ gelling system, the gel strength is important in finding the condition, which can delay the post nasal drip or anterior leakage. The gel strength was found to be affected by concentrations of gelling and bio adhesive polymers. The gel strength of the formulation was observed that the optimum concentration of formulation B2 (38.34.±0.03) sec., B4 (43.94±0.17) sec, and B6 (61.26±0.15) sec.

Mucoadhesive strength was determined in terms of detachment stress. All formulations were subjected to in vitro mucoadhesion test. The mucoadhesion force is an important parameter for in situ gelling nasal formulations since it prolongs the nasal clearance of gels and increases its residence time in nasal cavity.The reinforcement of the mucoadhesive forces in the nasal in situ gels by the use of mucoadhesive polymers could be explained by the fact that secondary bond forming groups (hydroxy, ethoxy and amine) are the principle source of mucoadhesion. Our study indicates that the variation in concentration of sodium alginate and xantham gum changes mucoadhesive strength. Figure 4 shows the effect of xantham gum and

sodium alginate on mucoadhesion strength. The significant effect was observed with xantham gum as compared to sodium alginate. This was due to wetting and swelling og gum, which permits intimate contact with nasal tissue, interpenetration of mucoadhesive xantham gum chains with mucin molecules leading to entanglement and formation of weak chemical bonds between entangled chains. Due to stronger mucoadhesive force, it can prevent the gelled solution coming out of the nose and increases its residence time in nasal cavity. But higher ratios are responsible for excessive mucoadhesive force and can damage nasal mucosa.

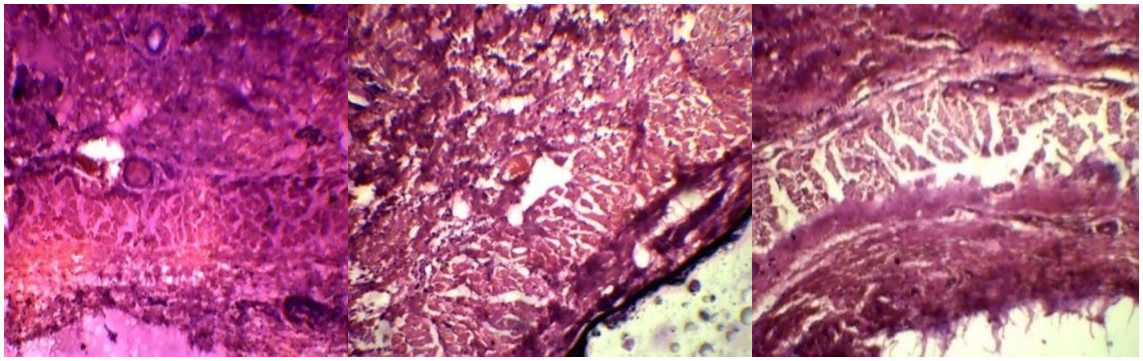
Fig 4: Mucoadhesive Foce (dyne/cm 2) of developed formulation



Photomicrographs of goat nasal mucosa after the permeation studies were observed for histopathological changes in comparison with the PBS treated mucosa. Histopathological evaluation of tissue incubated in Phosphate buffer saline 6.8 after collection it was compared with tissue incubated in the diffusion chamber with gel formulations (B3, B6).Tissue was fixed in 10% buffered Formalin (pH 7.2), routinely processed and embedded in paraffin. Paraffin sections (7 µm) were cut on glass slides and stained with hematoxylin and eosin. Sections were examined under a light microscope, to detect any damage to the tissue.

The section of nasal mucosa treated with formulation B3, B6 showed very slight degeneration of nasal epithelium along with no erosion. There was no sign of remarkable destructive effect of formulations on the treated nasal mucosa. Safety of a nasal formulation can be assessed by a comparative histological study of nasal mucosae after the ex vivo study and normal mucosae (control). The histological graphs (Fig. 5) revealed no significant alteration of nasal epithelium and/or necrosis occurred. This implies that Pluronic F127 is as safe as sodium alginate and xantham gum to be used as a mucoadhesive agent for nasal administration, and the pluronic concentration of used is also safe.

Fig 5: Photomicrograph of goat nasal mucosa used in mucosal toxicity study



PBS Treated **Formulaton B3** **Formulation B6**

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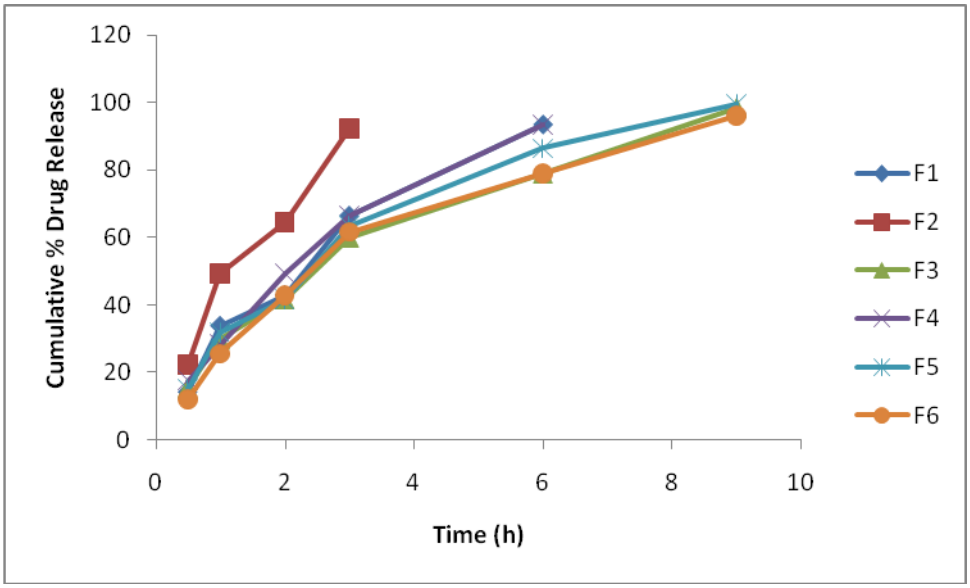
The ex-vivo permeation study showed that the formed gels have the ability to control the release of levetiracetam for the duration of about 540 minutes. The initial rates of drug release were very rapid due to incomplete gel formation, but as the time progresses the release rate decreases due to complete gel formation. With increase in concentration of PF127 along with sodium alginate were found to control the release gradually. The release profiles exhibited an inflection point, which indicated the gel formation in the donor compartment of diffusion cell. During gel formation, a portion of drug might be loaded into the gel matrix, thus the cross linking of polymer reduces the drug release rate. The initial rapid release of Levetiracetam was may be due to formation of prehydrated Matrix containing water filled pores due to presence of aqueous vehicle. In vitro release study indicated that the release of drug varied according to the type and concentration of polymers utilized. The results further showed that the amount of the drug released in 30 min decreased with the increasing polymer concentration and this pattern continued till the entire duration of study. The drug release profile was tested in phosphate buffer solution pH 6.8 as diffusion media. It was observed that pluronic F127 B3 (18%w/v) showed a release of drug 98.29 % at the end of 9 h. In vitro diffusion drug release of formulation containing various concentration of xantham gum B6 (0.3%w/v) was found to be 96.27 %.

Fig 3: In vitro Permeation Study of Developed Gel Formulations



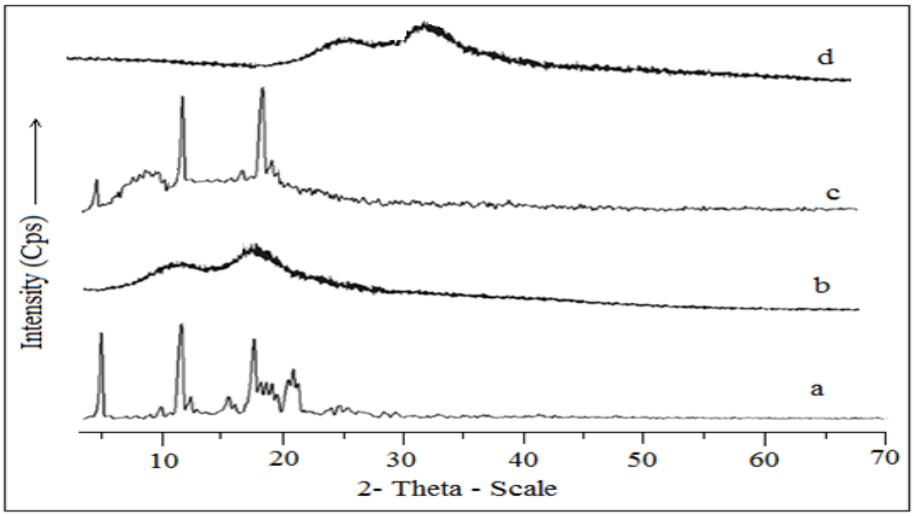


Fig 1: X – Ray Diffractogram of (a) Levetiracetam, (b) Drug/Sodium Alginate, (c) Drug/PluronicF127/SA physical mixture, (d) Xantham Gum

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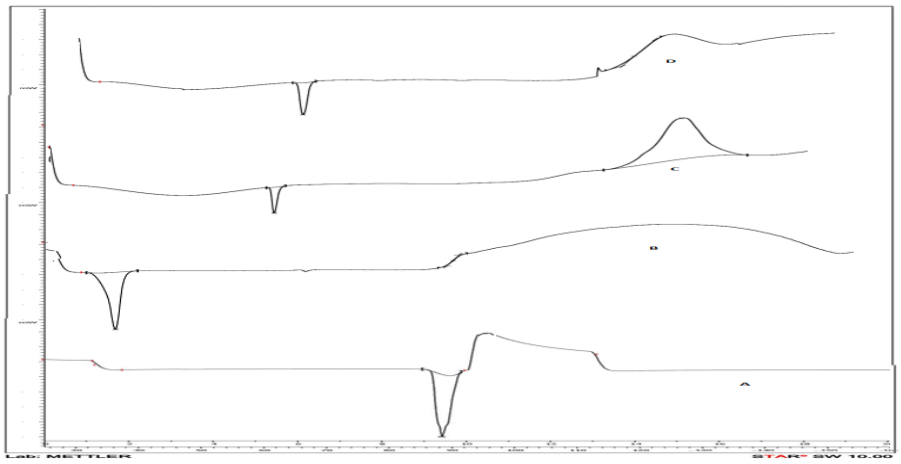

Fig 2: DSC spectra for: (a) Levetiracetam, (b) Drug + PluronicPhysical mixture, (c) Drug+Sodium alginate Physical Mixture. (d) Drug +Xantham Gum of Physical Mixture.

When the data is plotted as cum. % drug release vs time, if the plot is linear then data obeys Zero-order kinetics; with slope equal to Ko. The release constant was calculated from the slope of the appropriate plots, and the regression coefficient (R^2) was determined. It was found that the in-vitro drug release of B3 formulation was best explained by zero order, as the plots showed the highest linearity (R^2 =0.998).

Table 4: Model fitting Drug Release Kinetic

Batch Code	Zero Order	Higuchi Model	First Order	Best Fit Model	'k' value	'n' value	R^2	Mechanism
B3	0.9988	0.860	0.944	Zero order	1.132	0.749	0.994	(Non-Fickain)
B6	0.9286	0.919	0.910	Zero order	1.108	0.769	0.995	(Non-Fickain)

The stability studies carried out on optimized formulation Batch B3 & B6 at 30±2°C temperature and 60±5% RH for 90 days. The formulation was showing good stability with no remarkable change in drug content, gelation properties and gel strength. The result of stability studies (table 5,6) shown that there were no significant change in visual appearance, clarity and gelations but drug content of the optimized formulation B2 % was slightly changed. There was evidance of all the optimized formulation showed good stability in storage period (90Days) at 30±2°C temperature and 60±5% RH.

Table 5: Stability Study of Optimized Batch (3)

Sr.No	Parameter	Storage period (Days) at 30±2°C temperature and 60±5% RH			
		0	30	60	90
1	Appearance	Clear White	Clear White	Clear White	Clear White
2	Drug content (%)	98.22 ± 0.13	97.60 ± 1.41	95.74 ± 1.27	93.92 ± 1.34
3	Gelation study	+ + +	+ + +	+ +	+ + +
4	Gel strength (s)	39..23 ± 1.02	38.12 ± 2.14	37.76 ± 1.75	35.43 ± 1.07
5	*In-vitro* drug permeation (%)	99.59	96.41	93.24	90.47

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Table 6: Stability Study of Optimized Batch (6)

Sr.No	Parameter	Storage period (Days) at 30±2°C temperature and 60±5% RH			
		0	**30**	**60**	**90**
1	Appearance	Clear White	Clear White	Clear White	Clear White
2	Drug content (%)	98.52± 0.12	97.74± 1.55	96.15± 1.27	94.67± 1.28
3	Gelation study	+ + +	+ + +	+ +	+ + +
4	Gel strength (s)	35.45 ± 2.08	34.32 ± 2.12	32.65 ± 1.38	30.54 ± 1.42
5	*In vitro* drug release(%)	93.25	91.22	88.43	85.54

DISCUSSION

From the aforesaid study it was concluded that formulations prepared from Pluronic F127 along with the use of sodium alginate and xantham gum offer the alternate route for administration of gel via nose to brain. The system will require less amount of dose, decrease the dosing frequency and dose related side effects of the drugs. The polymer Pluronic F127, sodium alginate and xantham gum have proved to be useful as thermosensitive gelling agent and mucoadhesive agent respectively. The formulation prepared from xantham gum was found to possess better mucoadhesive properties than the sodium alginate that are widely used in preparation of nasal gels. In vitro permeation study showed that the drug was released up to 98. 29 % at the end of 9 h, in a controlled fashion. Histopathological study confirmed that these natural biopolymers had no adverse impact on the structural integrity of the nasal mucosa. Thus the formulation of in situ gelling system of Levetiracetam showed controlled release of drug, good stability and mucosal safety characteristics makes it more reliable, acceptable and beneficial for epileptic seizure treatment than other conventional dosage forms.

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REFERENCES

[1] Misra A, Kher G. Drug delivery systems from nose to brain. Curr Pharm Biotechnol. 2012;13(12):2355-79.
[2] More BA, Mene HR, Pawar RK, Misal NS, Pathak SS, Shivsharan KJ. A Review on In-Situ Nasal Gel Drug Delivery System. Int J Pharm Sci Rev Res. 2015; 33(1):199-207.
[3] Bassel Abou-Khalil. Levetiracetam in the treatment of epilepsy Neuropsychiatr Dis Treat. 2008; 4(3): 507–523.
[4] Sonmezturk HH and Azar NJ. Levetiracetam Extended Release as Adjuvant Therapy for the Control of Partial-onset Seizures. J Cent Nerv Syst Dis. 2011; 3: 17–25.
[5] Barnett EM, Cassell MD, Perlman S. Two neurotropic viruses, herpes simplex virus type 1 and mouse hepatitis virus, spread along different neural pathways from the main olfactory bulb.Neuroscience. 1993;57(4):1007-25.
[6] More PK, Saudagar RB and Gondkar SB. Nasal in-situ gel: a novel approach for nasal drug delivery system.World Journal of Pharmaceutical Research. 2015;4 (2): 686-708.
[7] Sambhaji SP, Anant RP, Kakasaheb RM, Shivajirao SK. Pluronic gels for nasal delivery of vitamin B12. Int J Pharm.2004 ; 270(1-2):37-45.
[8] Sharma N, Sharma A, Sharma PK, Garg G. Mucoadhesive Thermoreversible nasal delivery system. J Pharm Research. 2010; 3(5): 991-997.
[9] Mahajan HS, Tyagi V, Lohiya G, and Nerkar P. Thermally reversible xyloglucan gels as vehicles for nasal drug delivery. Drug Delivery. 2012;19(5):270–276.

[10] Basu S and Bandyopadhya AK.Development and Characterization of Mucoadhesive In Situ Nasal Gel of Midazolam Prepared with Ficus carica Mucilage**.** AAPS PharmSciTech. 2010; 11(3): 1223–31.
[11] Sherafudeen SP and VasanthaPV. Development and evaluation of in situ nasal gel formulations of loratadine. Res Pharm Sci. 2015; 10(6): 466–476.
[12] Gorle AP, Gattani SG. Design and Evaluation of Polymeric Ocular Drug Delivery System. Chemical & Pharmaceutical Bulletin 2009;57(9):914-19.
[13] Nerkar PP, Gattani SG. Oromucosal delivery of venlafaxine by linseed mucilage based gel: in vitro and in vivo evaluation in rabbits. Archives of Pharmacal Research. 2013;36(7):846-853.
[14] Singh RM, Kumar A, Pathak K. Thermally triggered mucoadhesive in situ gel of loratadine: ß-cyclodextrin complex for nasal delivery. AAPS Pharm Sci Tech. 2013;14:412–424.
[15] Mahajan HS, Gattani SG. Gellan gum based microparticles of metoclopromide hydrochloride for intranasal delivery: development and evaluation. Chem Pharm Bull. 2009;57:388–392.
[16] Jug M, Bećirević-Laćan M. Influence of hydroxypropyl-ß-cyclodextrin complexation on piroxicam release from buccoadhesive tablets. Eur J Pharm Sci. 2004;21:251–260.
[17] Majithiya RJ, Ghosh PK, Umrethia ML, Murthy RSR. Thermoreversible- mucoadhesive gel for nasal delivery of sumatriptan. AAPS Pharm Sci Tech. 2006;7:E80–E86.