DESIGN AND OPTIMIZATION OF LAMOTRIGINE IN-SITU GEL FOR BRAIN TARGETTING VIA NASAL DELIVERY THROUGH CENTRAL COMPOSITE DESIGN

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Abstract

The current study explores Lamotrigine for nasal delivery by incorporating the drug into a natural in-situ gelling system and further the retention in the nasal cavity was increased using the natural mucoadhesive polymer locust bean gum (LBG). The preliminary study was carried out to optimize the gellan gum concentration. The dose of the drug was estimated using the Robinson Erikson equation. The central composite design was engaged for optimization of the effect of individual variables gellan gum and locust bean gum on responses such as gelation time, the viscosity of the gel, mucoadhesive strength, and time taken for the drug to release half of its initial concentration (t_{50}).The drug and polymer compatibility was characterized by FT-IR and DSC. After evaluating all the parameters, formulation F6 which has 0.45%w/v gellan gum and 1.58%w/v LBG is selected as the optimized formulation.For F6 the drug release was sustained upto 12h (89.03%) with an adequate mucoadhesive strength.

Key words: Lamotrigine, gellan gum, locust bean gum, nasal delivery, brain targeting.

1. INTRODUCTION

International league against epilepsy (ILAE) defined epilepsy as a condition characterized by recurrent seizures unprovoked by any immediate identified cause [1]. Almost 50 million people are suffering from epilepsy among them 80% are from developing countries, the statement was made by WHO [2], this shows the emerging condition of epilepsy. Though Epilepsy is a CNS disorder, it affects social, physiological and vocational function along with brain physiology [3]. Many antiepileptic drug formulations are available in the market but they are limited to oral, transdermal, intravenous. One-third of the epileptic patients are still experiencing seizers and suffering from unaccepted medication-related side effects [4].The challenge accept in the treatment of epilepsy is to achieve seizure freedom without drug toxicity and affecting normal brain function. About 30% of epileptic patients experiencepharmacoresistance. The mechanism for the pharmacoresistance is still cagey [5]. So polytherapy came into existence and the combination of sodium channel blocker with GABA-ergic enhancer is the most effective polytherapy [6]. This polytherapy leads to transport of antiepileptic drugs concentration in high limiting to the effective drug concentration to the therapeutic target due to the overexpression of various efflux transporters [7].

Lamotrigine (LTG) is a novel and widely used second-generation antiepileptic drug in both partial and generalized seizures in adults and pediatrics alone or as adjunctive therapy [8]. Because of the P-glycoprotein mediated drug efflux, it cannot reach therapeutic concentration thereby doesn't show any effects in refractory epilepsy [9]. Presently Lamotrigine is available only as a tablet dosage form for oral delivery and because of the poor water solubility; the parental formulation was not available [10]. When a patient is emesis, undergoing any surgical treatment, or experiencing status epilepsy, LTG therapy cannot be continued but simultaneously termination of the LTG therapy leads to the increased risk of high seizure's activity [11]. To show the therapeutic effect, LTG should cross the blood brain barrier (BBB) with extensive systemic absorption. Therefore an attempt was made by increasing the dose of LTG, but this leads to the deposition of the drug concentration in blood and results in undesirable adverse effects like extreme skin rashes. So the alternative route of administration is demanded to control the partial and generalized seizures when the oral route is not realistic. In years back, nasal delivery is one of the prominent route than other routes for drug delivery as it is non-invasive, the patient can administer the drug by him/herself and more patient compliance [12]. Rapid onset of action is possible as absorption of drugs is more because of the high membrane permeability and rich vascularized nasal cavity [13]. At present, the vast research work is going on nasal in-situ gel dosage forms of CNS drugs where the drug is directly targeted to the brain.[14, 15].

In-situ gels are the types of gels that are in liquid form and convert to gel instantly after applying to the site of application through different mechanisms like temperature-induced, p^H triggering, by chemical reactions like ionic cross-linking and enzymatic cross-linking [16]**.**Numerous polymers are available for the in-situ gel formulations but the present research work was focused on biodegradable polymers which are easily and cheaply available. Among the polymers used for in-situ gels, Gellan gum is one of the ideal polymer.

It is an anionic deacetylatedexocellular polysaccharide secreted by the bacteria pseudomonas elodea [17,18]. When formulation consists of gellan gum administered at the site of application, it forms a clear gel upon contact with the cations present in the nasal secretions because of the cross-linking reaction of polysaccharide helices by the Na^+, K^+, and Ca^{+2} ions. This gelation delays the runoff process and prolongs the drug residence time. So in the present research work,gellan gum was selected as the in-situ gel polymer. Locust bean gum (LBG) is a natural polymer obtained from the seeds of leguminous plant *C.Siliqua* Linn, Fabaceae family.so it also called as carob bean gum. It is massively used as a food additive and a recognized scarcity of toxicity. So the use of locust bean gum was extended to pharmaceutical and biotechnological fields [19, 20, 21].Based on the literature, locust bean gum was selected as the mucoadhesive polymer in in-situ gel formulation through nasal delivery. Central composite design (CCD) is a statistical experimental design employed to determine the effects of experimental factors and related interactions with fewer experimental runs compared to other designs.

Though many papers were publishedregarding the Lamotrigine intranasal in-situ gel, none of them are focused on locust bean gum as a mucoadhesive polymer. Mucociliary clearance is one of the most considering factors in formulation design to nasal delivery.Hence the current research was aimed at the development of intranasal in-situ gel of Lamotrigine using gellan gum as an in-situ gelling agent and locust bean gum as mucoadhesive polymer. PEG 6000 was used as a permeation enhancer and benzalkonium chloride as a preservative. The central composite design was employed to study the effects of independent variables gellan gum (%w/v) and locust bean gum (%w/v)on responsesgelation time, the viscosity of the in-situ gel, mucoadhesive strength, and time is taken for the drug to reduce to the half of its initial concentration during*in-vitro* diffusion studies.

2. MATERIALS AND METHODS

Lamotrigine was a gift sample kindly supplied by CTX life sciences Pvt. Ltd. Gujarat, India. Gellan gum was kindly gifted by Marine Hydrocolloids, Cochin, India. Locust bean gum, PEG 6000, benzalkonium chloride was purchased from Merck life sciences PvtLtd.Methanol, Propylene Glycol, Acetone, Acetic acid, Dimethyl formamide, Potassium dihydrogen phosphate, Sodium hydroxide was purchased from SD Fine chemical Ltd.

2.1 Preformulation studies

2.1.1 Solubility studies:

To examine the solubility of Lamotrigine, excess drug was added in different organic solvents like water, methanol, propylene glycol, acetone, acetic acid, dimethyl formamide, phosphate buffer solution p^H 6.8 and 7.2. The 10ml solution of each flask wasequilibrated by shaking upto 48 hours on a thermostatically controlled water bath at 37±0.5^{o}C. After 48 hours the aliquots were filtered and analyzed spectroscopically at 303nm. [22, 23].

2.1.2 Melting point and λ_{max} determination

Melting point is one of the tests which can access the purity of the sample. The melting point of Lamotrigine was determined using the fusion method. Absorption maxima was determined in the phosphate buffer solution (PBS) p^H 6.4. The solution was scanned from 400 -200 nm using a UV-Visible spectrophotometer against PBS as a blank. [24, 25].

2.1.3 Fourier Transform Infra-Red Spectroscopy (FT-IR) Studies:

To evaluate the physical interactions between pure drug Lamotrigine and excipients used in the formulation, compatibility studies are carried out using FT-IR (Bruker Alpha T, Switzerland). The FT-IR of pure drug and excipients, alone and in combinations (1:1) was analyzed by the KBr pellet technique [26, 27].

2.2 Preliminary experiments for the selection of most suitable concentration range of gellan gum [28,29]

Gellan gum at different concentrations were prepared from 0.1 to 1%w/v using water as a solvent to access the critical cation concentration (CCC) of gellan gum for in-situ gel formulation. In the current study, CCC is measured in terms of the degree of gelation (time and stiffness of gel) were noted in grades form. The results were tabulated in Table 1.

Table 1: Gelation of gellan gum

S.No	Concentration of gellan gum(%w/v)	Grade
1.	0.1	-
2.	0.2	+
3.	0.3	++
4.	0.4	+++
5.	0.5	+++
6.	0.6	+++
7.	0.7	+++
8.	0.8	++++
9.	0.9	+++++
10.	1.0	+++++

2.3 Experimental design

The central composite design was employed for constructing a quadratic model for optimization of Lamotrigine in-situ gel keeping 2 independent variables and 4dependent variables using design Expert (version 12.0, state-Ease Inc., Minneapolis, Minnesota). The dependent and independent variable are as listed in Table 2. The quadratic equation generated by the design is as follows:

$$Y=\beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_3 X_1^2 + \beta_4 X_2^2$$

Where Y is the measured response of dependent variables, β_0 is the intercept, β_1 to β_4 are the regression coefficients computed from the observed experimental values Y. X_1 and X_2 are the coded values of the independent variables. X_1X_2 and X_1^2, X_2^2 represents interaction and quadratic terms respectively.

Table 2: Independent and dependent variables in central composite design

	Levels				
	-α	**-1**	**0**	**+1**	**+α**
Independent variables					
X_1=Gellan gum (%w/v)	0.096	0.2	0.45	0.7	0.8
X_2=Locust bean gum (%w/v)	1.59	2.0	3.0	4.0	4.41

Dependent variables	**Constraints**
Y_1= Gellation time (sec)	Minimum
Y_2= Viscosity of gel (cps)	Optimum
Y_3= Mucoadhesive strength of gel (dynes/cm^2)	Maximum
Y_4= *Invitro* $t_{1/2}$ (h)	Maximum

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2.4 Calculation of theoretical drug release profile

The total dose of LTG required for the in-situ gel was calculated as per the Robinson Erikson equation using the available pharmacokinetic data. The total dose D_t required is the summation of immediate D_i and maintenance dose D_m. $D_t=D_i + D_m$. Elimination rate constant $K=0.693/t_{1/2}$ =0.0288mg/h($t_{1/2}$ of the drug is 24 hours). Availability rate R=KD=0.7218mg/h, where D is the usual dose of the drug (25mg). D_m=Rh=8.662mg, h=Number of hours for which sustained action is desired (12h). D_i=D-RT_p=22.11mg, T_P=Time period to achieve peak plasma level (4h). D_t=30mg. so as per the Robinson Erikson equation, the formulation should release 22mg in the first 3-4 hour like conventional dosage forms and the remaining drug should be sustained upto 12h.[30, 31]

2.5 Preparation of Intra nasal *in-situ* gel

Gellan gum and LBG solutions were prepared individually simply by dispersing an accurate weighed quantity of gellan gum in hot distilled water using a magnetic stirrer for about 20 minutes. After complete dissolution, the LBG solution was added to the Gellan gum solution drop by drop with constant stirring. By keeping gum solution aside until it cools to 40^{o}C, meanwhile drug solution (30mg/ml using methanol (2-3 drops) and excipients solution was

prepared. PEG6000 and benzalkonium chloride were mixed by adding appropriate quantities in adequate water. This excipients solution was added to the gum solution with continuous stirring and make upto the final volume with distilled water. The formulations were filled in amber-colored glass vials containing rubber closures and sealed with aluminum caps. The vials were stored in the refrigerator (4-8^{o}C) until further use [32].

2.6 Evaluation of in-situ gels

2.6.1 Homogeneity and clarity studies, measurement ofp^{H}

The homogeneity and clarity of all the formulations were examined by visual observation against the white and black backgrounds for appearance and any particle existence. p^{H} was determined using standard p^{H} meter[33].

2.6.2 Gelation study

Gelation time

Gelation time is the time required for the liquid to convert from sol to gel form. The cations present in the gellan gum are responsible for the gelation. 2ml of each formulation was taken to the 10ml transparent vial containing a small amount of simulated nasal fluid (SNF) aqueous solution containing 8.77 mg/ml NaCl, 2.98 mg/ml KCl and 0.59 mg/ml $CaCl_2$ per liter). The vial was subjected for the stirring using a magnetic stirrer at 37±0.5^{o}C, gelling time is the time at which the magnetic bead was stopped stirring due to gelation.

Gel strength

Gel strength is the mark of the viscosity of formulation in physiological conditions. It is measured in terms of time. The time is taken for the 3.5 g of weight to penetrate into the depth of about 3cm in a 5 g in-situ gel. [34, 35]

2.6.3 Syringiblity

Syringibility test is the indication for the passage of formulation from the dosage container to the application site. The test was performed by taking the 1ml of formulation to the 5ml of syringe consisting of a 20 gauge needle. If the solution is easily passed from the syringe termed as ‘pass’ and vice versa. [36]

2.6.4 Viscosity Studies

Viscosity is measured to ensure the flow, quality, and consistency of the formulation. The viscosity of all the formulations before and after gelation was measured using a digital Brookfield viscometer fitted with an S-63 spindle at 100rpm. [37]

2.6.5 Spreadability

The test was performed to evaluate the spreading of the gel on the physiological membrane. The excess amount of sample (0.7g gel) was sandwiched between the two slides. The upper slide was attached to the 20g weight. 100g of weight was placed on the upper slide for 5 minutes to ensure the uniform distribution of the sample, the excess of the gel was wiped using the tissue at the edges. As soon as the 100g weight was removed, the time taken for the upper slide to slip off from the bottom slide is noted and Spreadability(S) was calculated using the formula S=W×L/T where W is the weight attached to the upper slide and L is the length of the slide 7.5cm. [38]

2.6.6*In-vitro* Mucoadhesion Strength

The *in-vitro* mucoadhesive strength was determined using a fabricated physical balance in the laboratory. The sheep nasal mucosa was used for the study. The mucosa was stored in the phosphate buffer p^H 6.5 until use. One side of the balance the rubber closure containing the nasal mucosa of area 1.33 cm^2 facing outwards was tied and another mucosa was placed to the outside of the beaker in such a way that the two mucosae face each other. A 50 mg of sample was placed on the mucosa of the beaker and allowed to remain in contact with each other for a few minutes. The other side of the balance weights are kept going to place until two mucosae detach from each other. The mucoadhesive strength dynes/cm^2=mg/A, where m is weight in grams required for the detachment of mucosae, g is the acceleration due to gravity and A is the area of the mucosa. [39]

2.6.7*In-vitro* drug release

In-vitro drug release studies were carried using the adjusted Franz diffusion cell. The dialysis membrane (12,000-14,000 kDa) which was previously soaked in the phosphate buffer p^H 6.5for 12 hours was mounted to the diffusion cell. The formulation equivalent to the 30mg of drug was placed in the donor compartment and SNF of about 50ml was placed in the receptor compartment. The temperature of 37±0.5^{0}C and 100 rpm was maintained throughout the experiment to mimic the biological conditions. At regular time intervals specified amount of sample was pipetted out and simultaneously replaced with the SNF of the same quantity to maintain sink conditions. The pipetted sample was diluted accordingly and analyzed using a UV spectrophotometer at 303 nm. [26]

2.6.8*Ex- vivo* studies

The sheep nasal mucosa which was carefully separated from the connective and cartilaginous tissue was used for the study. The mucosa of surface area 3.8 cm^2 was clutched between the donor and receptor compartment. 50ml of SNF was taken into the receptor compartment. The drug equivalent to 30 mg of Formulation F2 and F6 was placed in the donor compartment after the pre-incubation of about 20 minutes. The same temperature, rpm, and sampling intervals were maintained as that of the *in-vitro* drug release study. The apparent permeability coefficient (cm/h) was calculated using the formula P_{app}=Q/A×c×twhere Q is the total amount of drug permeated during the incubation (µg), A is the area of the diffusion cell (cm^2), c is the initial concentration of drug taken into the donor compartment and t is the total time taken for

complete release of the drug. The graph was plotted for cumulative percent of drug permeated against time. [40]

2.6.9 Differential scanning calorimetry (DSC)

DSC of the pure drug and physical mixture of drug and excipients (stored at 37±2^{o}C, relative humidity RH 75±5% for 2 months) were performed using (Mettler Toledo, Japan). The sample of about 10mg was sealed in aluminum pans and scanned at a heating rate of 10^{o}C per minute over the temperature range of 30 to 300^{o}C for drug and 30 to 300^{o}C for the physical mixture. [41]

2.6.10 Stability studies

The intention to do the stability studies is to bestow the confirmation on what way the quality of the drug formulation varies with the time under the influence of the factors like temperature, humidity, and light. The study helps to exhibit the shelf-life of the formulation and to endorse the storage conditions. The abundant quantity of in-situ gel was sealed in the amber-colored vials and stored at relative humidity, the temperature at 75±5% and 40±2^{o}c respectively. The samples for the estimation of clarity, *in-vitro* gelation time, drug content, and drug release were withdrawn at time intervals of 1, 2, 3 months.

3. RESULTS AND DISCUSSION

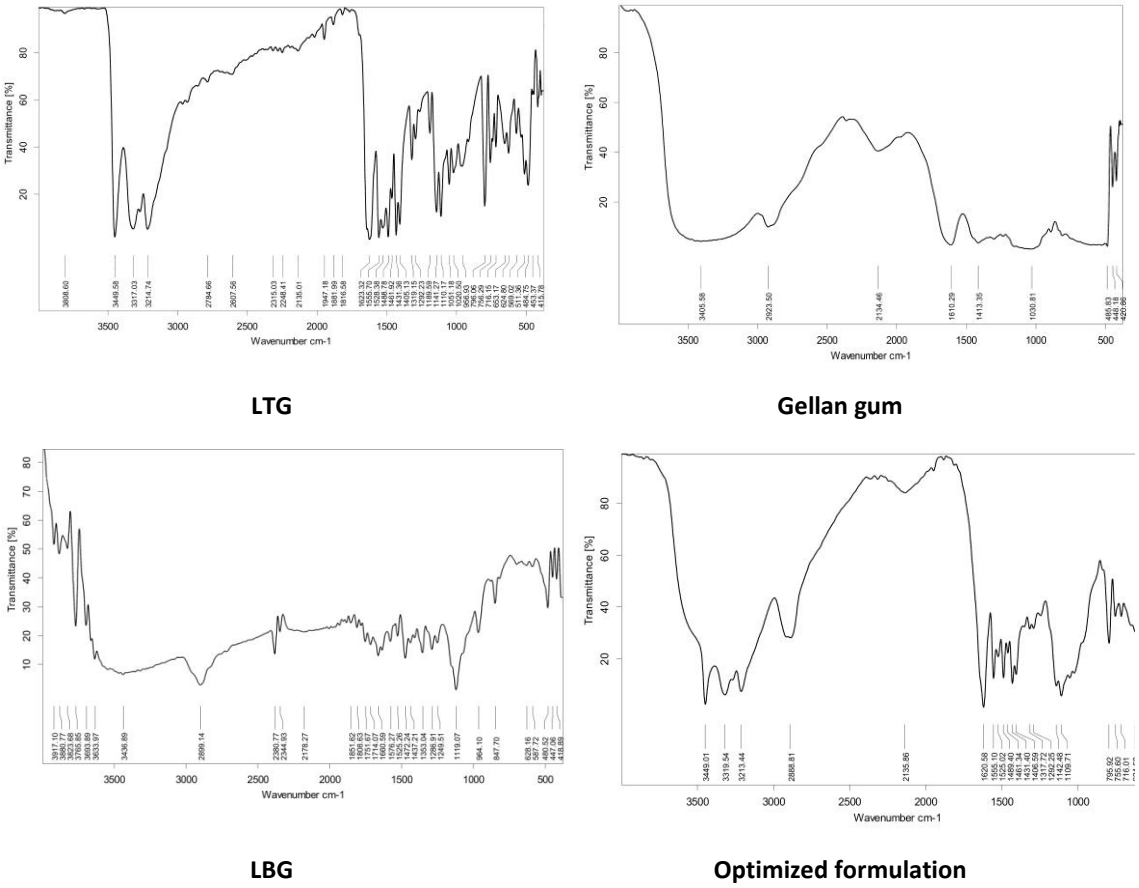
3.1 Solubility, Melting point

The pure drug Lamotrigine was found to be slightly soluble in methanol (1.73mg/ml), propylene glycol (1.78mg/ml), and acetone (1.67mg/ml). It was very slightly soluble in PBS p^{H} 6.8 (0.34mg/ml) and 7.2 (0.4mg/ml) whereas freely soluble in dimethylforamide (125.36mg/ml). 0.275mg/ml solubility was found in water which indicates the drug is very slightly soluble in water. The solubility terms used were based on the Indian pharmacopeia IP.The melting point was found to be 216-218°C, in accordance with that of reference.

3.2 Fourier Transform Infra-Red Spectroscopy (FT-IR) Studies

The FT-IR spectra of pure drug LTG, gellan gum, Locust bean gum, PEG 6000, and physical mixture of all the above components are shown in (Fig.1). The pure drug sample showed the intense peaks 3449.58 cm^{-1}, 3317.03 cm^{-1}, 1623.32 cm^{-1}, 1431.36 cm^{-1} and 796.06 cm^{-1} which are due to the N-H stretching at aliphatic primary and secondary amine, C=C stretch of alkene, C-H bending of alkane, and C-Cl stretch of halo compound respectively. Finally, the formulation, the physical mixture of pure drug, gellan gum, locust bean gum, and PEG 6000 exhibited the intense peaks at the wavelengths of 3449.01 cm^{-1}, 3319.54 cm^{-1}, 3213.49 cm^{-1}, 2888.81 cm^{-1}, 1620.58 cm^{-1}, 1431.40 cm^{-1}, 1109.71 cm^{-1} and 795.92 cm^{-1}. This data validates that there was no major shifting in the functional groups of pure drug, individual samples of excipients, and physical mixture of drug and excipients. Thus concluded that there is no incompatibility between the drug and excipients which could affect the chemical stability of the in-situ gel.

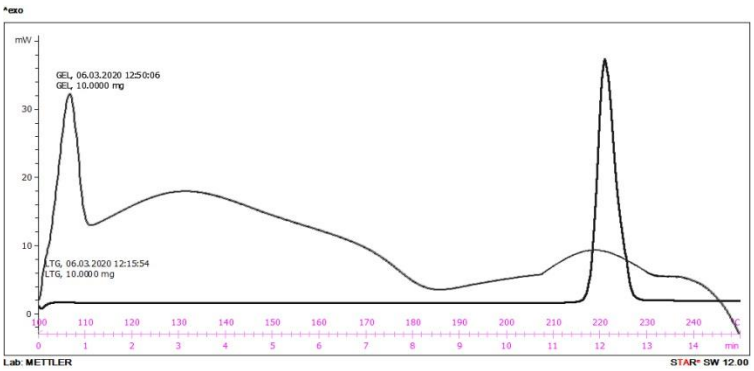
Fig.1: FT-IR graphs of pure drug, excipients and optimized formulation



3.3 Differential scanning calorimetry (DSC)

The DSC thermograms of pure drug and the physical mixture were shown in (Fig.2). The pure drug exhibited a sharp exothermic peak at 223.01^{o}C. The physical mixture showed the board exothermic peak at 216.15^{o}C which confirms that the formulation the chemical stability and some additional board peaks appeared at 58.6^{o}C, 84.6^{o}C due to the presence of excipients.

Fig. 2: DSC of the pure drug and formulation



3.4 Selection of polymer concentration

The concentration of gellan gum was selected from 0.2 – 0.7%w/v based on the degrees of gelation shown in Table 1. Less than 0.2%w/v the polymer is doesn't convert from sol to gel and beyond the 0.7%w/v, a hard instant gel was formed which is not suitable for the formulation for the penetration of the drug. The concentration of Locust bean gum was selected as 2–4 %w/v, which is the most suitable mucoadhesive concentration.

3.5 Experimental design

The selected four dependent variables showed a varied difference in all the 13 batches as shown in Table 3. For deriving the conclusion, the polynomial equations are used after considering the magnitude and sign of the coefficients.

Table 3: Results of the experimental design

Formulation	X1 (%w/v)	X2 (%w/v)	Y1 (Sec)	Y2 (Cps)	Y3 (dynes/cm2)	Y4 (Hours)
F1	0.45	3	6.66±0.57	137±3.3	2514.94	1
F2	0.45	4.41421	8±1.0	151.2±7.0	2741.65	1
F3	0.7	4	3.33±0.34	261.98±2.59	3017.68	1.5
F4	0.45	3	6.33±0.57	134.8±3.49	2514.94	1
F5	0.45	3	6.66±1.15	136.77±3.02	2514.94	0.8
F6	0.45	1.58579	5.33±0.53	133.15±1.79	2318.59	1.5
F7	0.803553	3	1.33±0.57	301.8±5.03	3574.14	1.25
F8	0.2	4	21.6±0.57	122.26±1.75	1147.24	1.5
F9	0.7	2	2.33±0.57	252.11±2.32	2846.12	1
F110	0.45	3	6.6±0.57	134.72±3.47	2514.94	1
F11	0.0964466	3	44±3.6	93.27±2.92	1042.61	8
F12	0.2	2	19±1.0	119.25±1.14	1078.48	0.83
F13	0.45	3	6.33±0.57	135.86±4.44	2514.94	1

*All the values are in mean±sd (n=3), X1=Gellan gum, X2=LBG, Y1=Gelation time, Y2=Viscosity of gel, Y3= t50%

3.6 Effect of the formulation variables on gelation time (Y1)

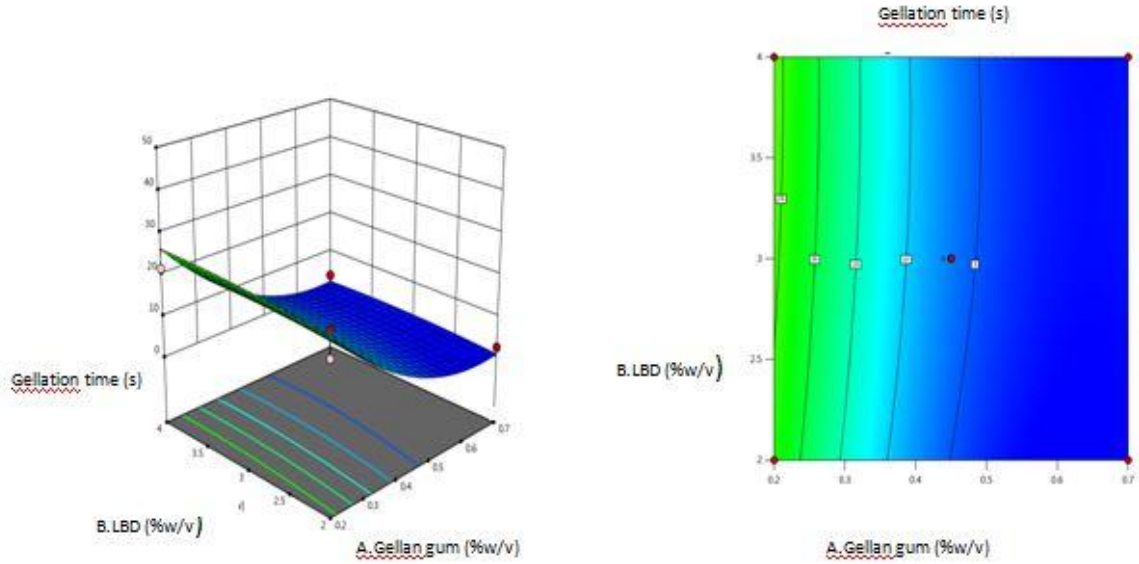
With respect to the response Y1, the outcome of the multiple regression analysis reveals that the coefficient β_1 bearsa negative sign and β_2 has a positive sign. This indicates that as the concentration of gellan gum increases the gelation time decreases. The β_2 has a positive sign that shows gelation time increases an increase in the LBG concentration. The fitted equation for the response Y1 to the transfigure factor is shown in the following equation.

$$Y1=6.52- 11.91X_1 + 0.9220X_2 - 0.4000 X_1X_2+ 7.30 X_1^2 - 0.7005 X_2^2$$

The Y1 for all the runs F1 to F13 shows a good correlation co-efficient of 0.8950. The variable X_1 has the P-value <0.0001 and X_2 has 0.5125. This indicates that only the concentration of gellan gum significantly affects the gellan gum but not the LBG as the P-

value for X_2 is >0.05. This is due to the interaction between the ions of the gellan gum and the concentration of the nasal secretions. As the gum concentration increases the ions of the gel also inclined this leads to less gelation time. The correlation between formulation variables and response Y1 was further illuminated using response surface and contour plot as shown in (Fig. 3).

Fig. 3: 3D Response surface and contour plot for the response Y1.



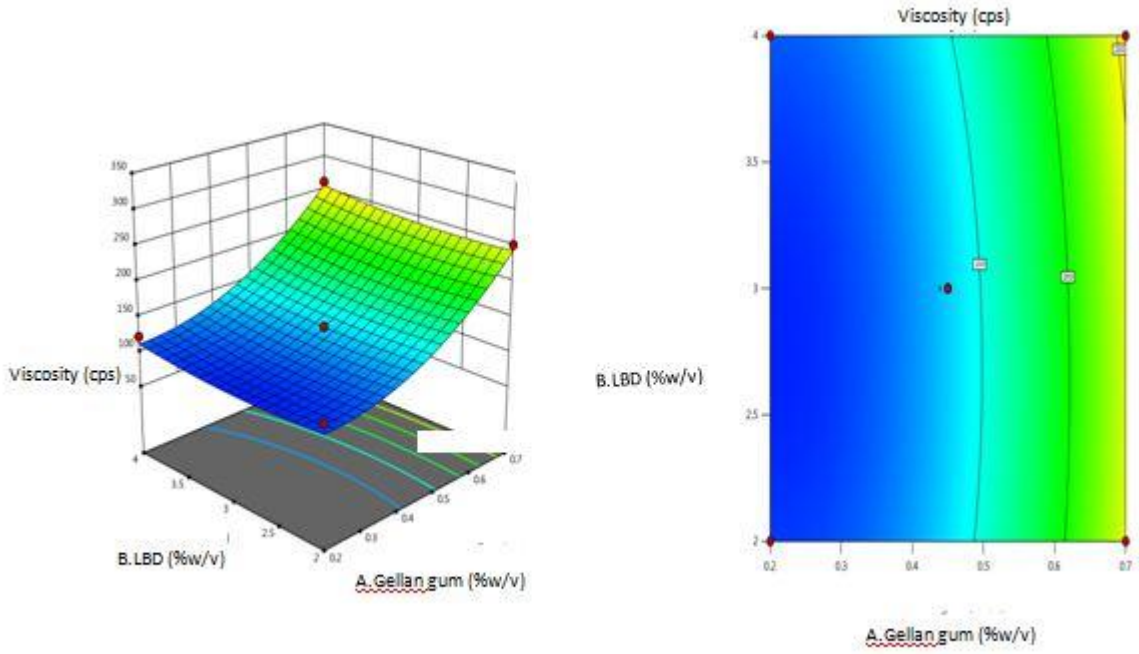
3.7 Effect of the formulation variables on viscosity (Y2)

On concerning the response Y2, the end result of the multiple regression analysis showed that both the coefficients β_1 and β_2 has a positive sign. This indicates that an increase in the concentration of gellan gum and LBG results in an increase of viscosity. The fitted equation for the response Y2 to the transfigure factor is shown in the following equation.

$$Y2=135.83+70.94X_1 + 4.80X_2+1.72X_1X_2+35.61X_1^2 +7.93X_2^2$$

The response Y2 for all the runs F1-F13 showed a good correlation coefficient of 0.9722. The variable X_1 has the P-value <0.0001 and X_2 has 0.2479 both the values are >0.05. This indicates that only X1 significantly affects the response Y_2 compared to X_2. Mostly in the nasal preparations the viscosity should be optimum in such a way that it should hold to the nasal mucosa until it absorbs. Table 3 also shows that as the concentration of gums increases the viscosity also increases but high viscous gels tenders the drug release. The interaction between formulation and response variable can be further exhibited by the 3D surface and contour plots as shown in (Fig.4)

Fig. 4: 3D Response surface and contour plot for the response Y2



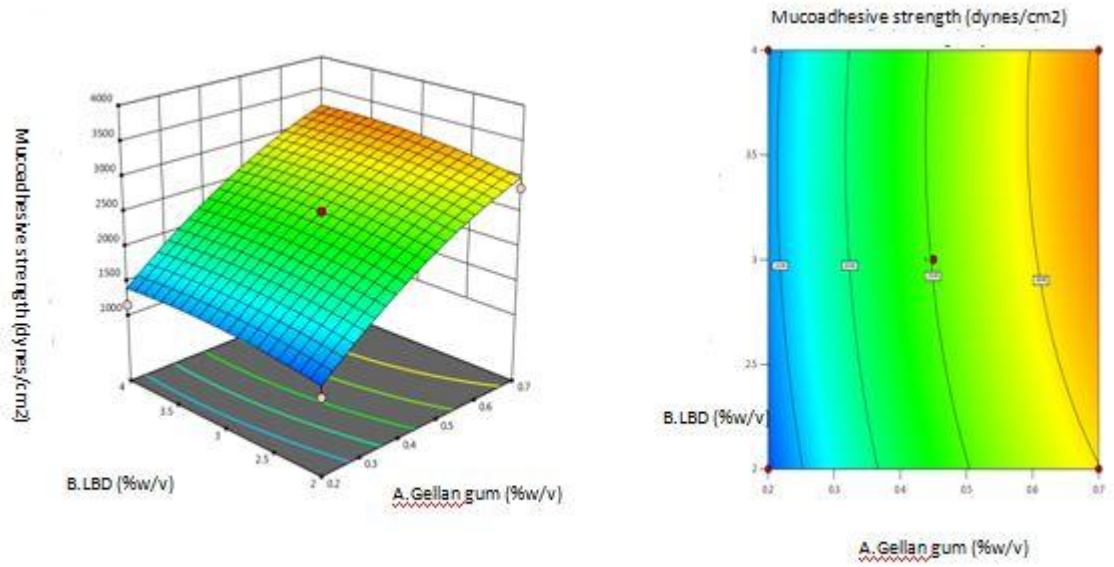
3.8 Effect of the formulation variables on mucoadhesive strength (Y3)

With respect to the response Y3, the results of the multiple regression analysis reveal that both the coefficients β_1 and β_2 possess positive sign. This indicates that the mucoadhesive strength increases with an increase in the concentration of gellan gum and LCB gum. The fitted equation for the response Y3 to the transfigure factor is shown in the following equation.

$$Y3=2514.94+902.28X_1 + 104.83X_2+25.70X_1X_2-202.50X_1^2 -91.63X_2^2$$

The response Y3 for all the runs F1-F13 showed a correlation coefficient of 0.8925. The variable X_1 has the P-value <0.0001 and X_2 has 0.0215 both the values are <0.05. This indicates that both variables X_1 and X_2 significantly affect the response Y3. As the concentration of gums increases with an increase in the viscosity the mucoadhesive strength also increases. The response is clearly exhibited in (Fig. 5).

Fig. 5: 3D Response surface and contour plot for the response



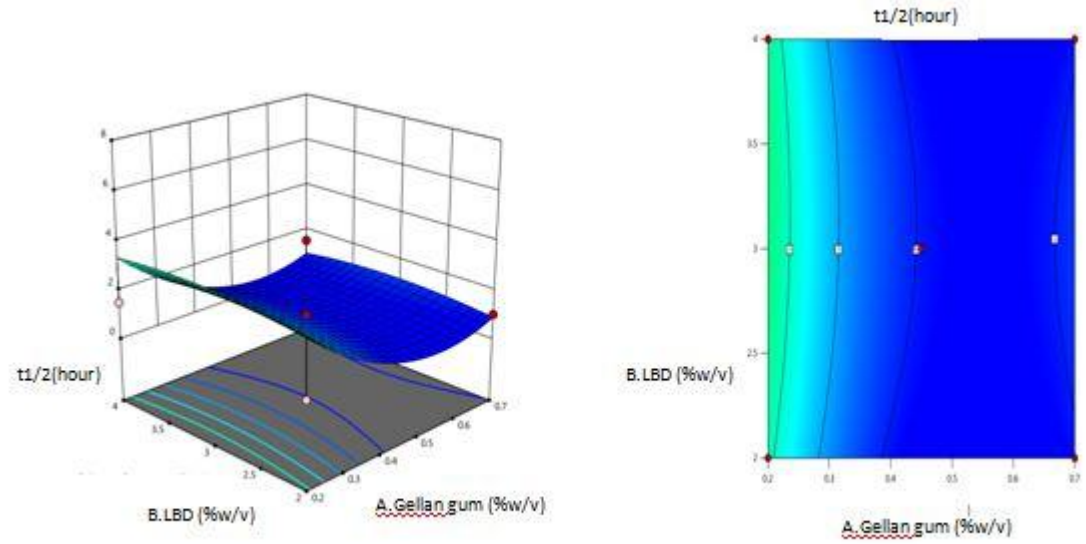
3.9 Effect of the formulation variables on the $t_{50\%}$ (Y4)

$t_{50\%}$ is the time taken for the drug release to reduce to its 50% of concentration. On concerning the response Y4, the end result of the multiple regression analysis showed that the coefficients β_1 negative and β_2 has a positive sign. The fitted equation for the response Y4 to the transfigure factor is shown in the following equation.

$$Y4=0.96-1.17X_1 + 0.0579X_2-0.0425X_1X_2+1.40X_1^2 -0.2875X_2^2$$

The response Y4 for all the runs F1-F13 showed a correlation coefficient of 0.2963. The variable X_1 has the P-value of 0.092 and X_2 has 0.9221. This indicates that the variable X_1 shows a significant impact on the $t_{50\%}$ than the X_2. As the concentration of gellan gum increases the drug release also increases but only to a certain level. Beyond the optimum concentration of gellan gum, the drug release decreases this is due to the hindrance of the drug molecules in the polymer cavities. This can be clearly elucidated in the 3D surface and contour plots as shown in (Fig. 6).

Fig. 6: 3D Response surface and contour plot for the response Y4



3.10 Homogeneity, clarity, p^H of in-situ gels

All the prepared formulations were clear without any coalesces in the sol form. The p^H of all formulations ranged from 5.84 – 5.9, which indicates a suitable range for nasal delivery without any irritation of the mucosa. The p^H range is also suitable for the solubility of the drug.

3.11 Gel strength, Syrengibility and Spredability

Gel strength plays an important role in the formulation of nasal in-situ gels. The good gel strength formulation allows the administration of droplets easily from the container and extends its post nasal drip. The formulations F1-F13 possess gel strength in the range of 1.02 to 30.51 sec. The F11 formulation has gel strength of 1.02 sec which is not suitable for the administration and F7 has 30.51 sec which creates discomfort upon the administration because of its gel stiffness. So the F1,F4,F5,F6,F10 and F13 formulations has the optimum gel strength. The Table 4 clearly exhibited that the syrengibility increases with increase in the concentration of gel. The formulations which are difficult to pass and very easily pass from the syringe are considered as fail in the test as they retard the drug release. So only passed formulations are taken to consideration. Among all the formulations only F6 has the spreadability of 2.61 g cm/sec which is adequate to increase the residence time.

Table 4: Results for p^H, gel strength, syringibility, spreadability, viscosity of sol, drug content

Formulation	p^H	Gel strength (sec)	Syringibility	Spreadability (g cm/sec)	Viscosity of sol (cps)	Drug content (%)
F1	5.58±0.0	8.13±0.54	Pass	26.50	30.62±0.19	90.9±0.65

	3					
F2	5.84±0.14	8.35±0.67	Pass	28.14	46.33±0.188	89.26±1.36
F3	5.97±0.45	24.67±0.87	Difficult to pass	64.37	80.04±0.46	79.50±2.04
F4	5.89±0.62	8.13±0.98	Pass	26.50	30.62±0.19	91.61±0.87
F5	5.34±0.56	8.15±0.81	Pass	26.50	30.62±0.19	91.26±0.97
F6	5.84±0.34	7.98±0.43	Pass	2.61	30.62±0.19	91.69±1.32
F7	6.12±0.61	30.51±0.52	Difficult to pass	150	94.55±0.29	78.92±2.79
F8	5.12±0.73	3.61±0.43	Pass	17.32	24.4±3.45	92.05±1.84
F9	5.39±0.87	23.48±0.67	Difficult to pass	56.39	74.33±0.45	79.97±2.12
F10	5.62±0.43	8.54±0.12	Pass	26.50	30.62±0.19	89.69±1.06
F11	5.27±0.69	1.02±0.22	Very easily Pass	7.89	9.85±0.26	94.87±1.32
F12	5.81±0.18	3.65±0.52	Pass	15.48	21.02±0.46	94.15±0.94
F13	5.32±0.25	8.56±0.67	Pass	26.50	30.62±0.19	93.00±1.73

3.12 Viscosity

The viscosity was measured both for sol and gel states. In both states the viscosity increases with an increase in the concentration of gellan gum. Based on the results from Table 4 and (Fig. 3), the formulation F6 has desired viscosity of 30.62 and 133.15 cps in sol and gel state respectively.

3.13 Drug content

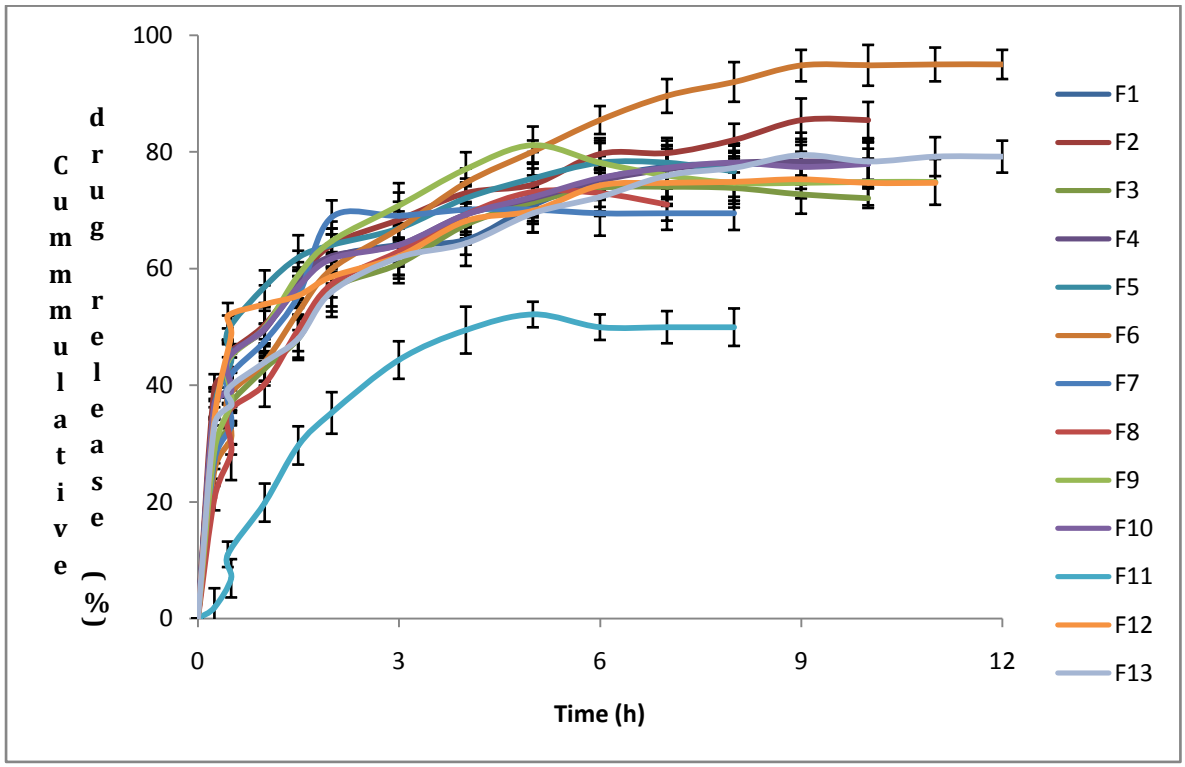
The drug content was ranged from 79.50-94.87%. The F6 formulation which has the 0.09%w/v gellan gum and 3%w/v LBG showed the highest drug content 94.87% and the F7 has the least drug content 79.50% which consists of 0.8%w/v and 3%w/v of gellan gum and LBG respectively. This shows that a linear relationship between the drug content and concentration of gellan gum.

3.14*In-vitro* Drug release

As shown in (Fig. 7), the effect of the polymer concentration in the drug release clearly exhibits that as the concentration of polymer increases the drug release also increases but only to a certain level. Beyond that, the decline in the cumulative drug release (CDR) is shown in formulations F7 and F9 because the more polymer concentration the drug

molecules are strongly gets imbibed in the gel structure. In formulations F8, F11 and F12 where the drug molecules are not strong interlinked with the drug molecules due to less polymer concentration, burst release was seen. The F2 formulation has a drug release of 85.46±3.1% at the end of the 10th hour. Among all the formulations the F6 shows the sustained drug release of 94.99±2.51% up to 12 hours. So the F2 and F6 formulations are selected for the *ex-vivo* studies.

Fig. 7: In-vitro drug release profile of formulations F1-F13
(All the values expressed are mean ±SD, n=3)



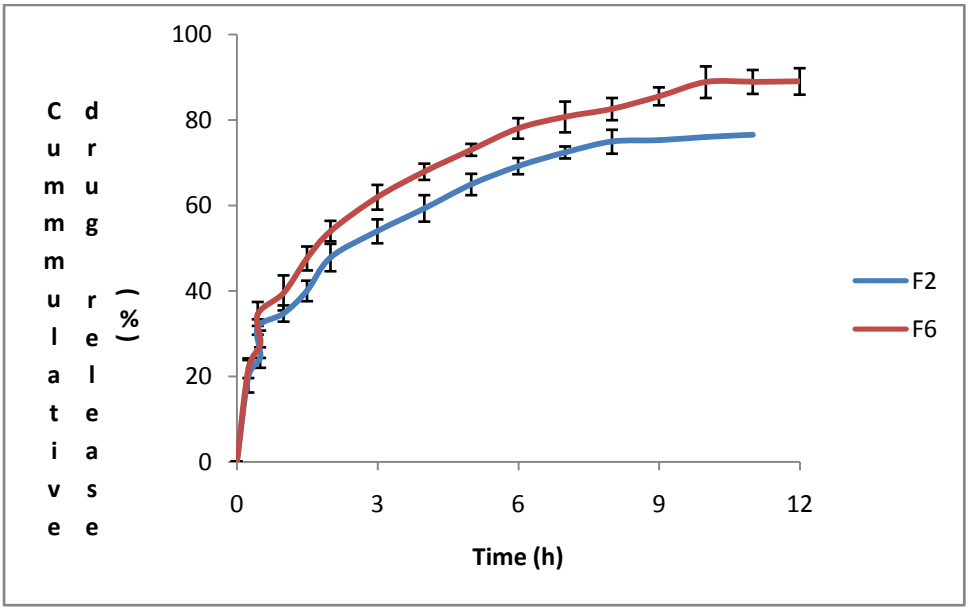
To determine the drug release mechanisim from in vitro drug release data obtained from the improved formulation, various kinetic models like zero order, first order Higuchi and Krosmeyer Peppas model were fitted. Both Higuchi and Krosmeyer Peppas models showed good linearity with regression R2 values 0.982 and 0.990 respectively which suggests that the release mechanisim was diffusion controlled.

3.15*Ex- vivo* studies

The *ex- vivo* studies were performed for the F2 and F6 formulations. Both the formulations showed a slighter CDR compared to the *in-vitro* CDR. F6 formulation showed 89.03±0.78% drug release at the end of the 12th hour and F2 shows 76.53±0.67% as shown in (Fig. 8). The apparent permeability coefficient (P_{app}) was found to be 0.015 and 0.018cm/s for F2 and F6 formulations respectively.

Fig. 8:*Ex- vivo* drug release profile

(All the values are expressed are mean ±SD, n=3)



3.16 Stability studies

The optimized formulation F6 was elevated for short term stability testing at 1st, 2nd and 3rd months. The results of the stability studies were given inTable 6. From the results it can be concluded that there is not much remarkable change in the clarity, gelation time,drug content and drug release.

Test parameter	Result		
	1st Month	2nd Month	3rd Month
Clarity	Clear	Clear	Clear
Gelation time (S)	5.8±1.5	5.6±0.8	6.4±2.1
Drug content (%)	90.2±1.7	90.0±2.6	87.62±0.6
CRP at 6h (%)	83.3±1.5	81.8±1	81.3±2.9
CRP at 12h (%)	93.5±3.2	93.1±2.5	92.6±2.9

Table 6 Results of stability testing

4. CONCLUSION

Based on results of the current study, the targeting potential of the drug can be enhanced by formulating the drug into intranasal in-situ gel. *In vitro* results indicate that formulation has optimum p^H, suitable for nasal mucosa with adequate mucoadhesive strength, ease of application, and residence time by the defeat of the mucociliary clearance.Theresultsof the studyindicate that natural polymers gellan gum and LBG has successfully performed their role in formulation design by in-situ gelation with adequate mucoadhesive strength. The

results explores intranasal in-situ gel is a prominent and safe drug formulation for epilepsy treatment compared to that of the oral route.

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All the authors have equally contributed to this manuscript.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

References

[1] Hauser WA, Kurland LT. The epidemiology of epilepsy in Rochester, Minnesota, 1935 through.Epilepsia. 1967 Mar;16(1):1-66. doi: 10.1111/j.1528-1157.1975.tb04721.x. PMID: 804401.

[2] Geneva: World Health Organization. WHO. Neurological Disorders: Public Health Challenges. 2006.

[3] Dudra-Jastrzebska M, Andres-Mach MM, Łuszczki JJ, Czuczwar SJ. Mood disorders in patients withepilepsy. Pharmacol Rep. 2007 Jul 1;59(4):369-78. PMID: 17901565.

[4] French JA. Refractory epilepsy: clinical overview. Epilepsia. 2007 Mar;48Suppl 1:3-7. doi: 10.1111/j.1528-1167.2007.00992.x. PMID: 17316406.

[5] Małek R, Borowicz KK, Kimber-Trojnar Z, Sobieszek G, Piskorska B, Czuczwar SJ. Remacemide--a novel potential antiepileptic drug. Pol J Pharmacol. 2003 Sep;55(5):691-8. PMID: 14704464.

[6] Kwan P, Brodie MJ. Early identification of refractory epilepsy. N Engl J Med. 2000 Feb 3;342(5):314-9. doi: 10.1056/NEJM200002033420503. PMID: 10660394.

[7] Löscher W. Drug transporters in the epileptic brain. Epilepsia. 2007 Mar;48Suppl 1:8-13. doi: 10.1111/j.1528-1167.2007.00993.x. PMID: 17316407.

[8] Goa KL, Ross SR, Chrisp P. Lamotrigine. A review of its pharmacological properties and clinical efficacy in epilepsy. Drugs. 1993 Jul;46(1):152-76. doi: 10.2165/00003495-199346010-00009. PMID: 7691504.

[9] Zhang C, Kwan P, Zuo Z, Baum L. The transport of antiepileptic drugs by P-glycoprotein. Adv Drug DelivRev. 2012 Jul;64(10):930-42. doi: 10.1016/j.addr.2011.12.003. Epub 2011 Dec 16. PMID: 22197850.

[10] Biton V. Pharmacokinetics, toxicology and safety of lamotrigine in epilepsy. Expert Opin Drug MetabToxicol. 2006 Dec;2(6):1009-18. doi: 10.1517/17425255.2.6.1009. PMID: 17125413.

[11] Liu JS, Wang JH, Zhou J, Tang XH, Xu L, Shen T, Wu XY, Hong Z. Enhanced brain delivery of lamotrigine with Pluronic(®) P123-based nanocarrier. Int J Nanomedicine. 2014 Aug 16;9:3923-35. doi:10.2147/IJN.S62263. PMID: 25152622; PMCID: PMC4140705.

[12] Illum L. Nasal drug delivery--possibilities, problems and solutions. J Control Release. 2003 Feb 21;87(1-3):187-98. doi: 10.1016/s0168-3659(02)00363-2. PMID: 12618035.

[13] Abouhussein DM,Khattab A,Bayoumi NA, Mahmoud AF, Sakr TM. Brain targeted rivastigminemucoadhesivethermosensitive *In situ* gel: Optimization, *in-vitro* evaluation, radiolabeling, *in vivo* pharmacokinetics and biodistribution. JDrug DelivSciTechnol.2018Feb;(43):129-140.

[14] Abdou EM, Kandil SM, Miniawy HM. Brain targeting efficiency of antimigrain drug loaded mucoadhesive intranasal nanoemulsion. Int J Pharm. 2017 Aug 30;529(1-2):667-677. doi: 10.1016/j.ijpharm.2017.07.030. PMID: 28729175.

[15] Trapani A, De Giglio E, Cafagna D, Denora N, Agrimi G, Cassano T, Gaetani S, Cuomo V, Trapani G. Characterization and evaluation of chitosan nanoparticles for dopamine brain delivery. Int J Pharm. 2011 Oct 31;419(1-2):296-307. doi: 10.1016/j.ijpharm.2011.07.036. PMID: 21821107.

[16] Singh RM, Kumer A, Pathak K. Mucoadhesive *in situ* nasal gelling drug delivery systems for modulatedDrugdelivery. Expert OpinDrug Delivery. 2013 Jan 1;10(1):115-30.doi: 10.1517/17425247.2013.746659.

[17] Dave PN, Gor A. Natural Polysaccharide-Based Hydrogels and Nanomaterials: Recent Trends and Their Applications. Handbook of nanomaterials for industrial applications.2018 Jan 1:36-66.

[18] Yadav H, Karthikeyan C. Natural polysaccharides: Structural features and properties. Polysaccharide carriers for drug delivery.2019;1-17.DOI - 10.1016/B978-0-08-102553-6.00001-5

[19] Prajapati VD, Jani GK, Moradiya NG, Randeria NP, Nagar BJ. Locust bean gum: a versatile biopolymer. CarbohydrPolym. 2013 May 15;94(2):814-21. doi: 10.1016/j.carbpol.2013.01.086. Epub 2013 Feb 11. PMID: 23544637.

[20] Dionísio M, Grenha A. Locust bean gum: Exploring its potential for biopharmaceutical applications. J Pharm Bioallied Sci. 2012 Jul;4(3):175-85. doi: 10.4103/0975-7406.99013. PMID: 22923958; PMCID: PMC3425165.

[21] Prajapati VD, Jani GK, Moradiya NG, Randeria NP, Maheriya PM, Nagar BJ. Locust bean gum in the development of sustained release mucoadhesive macromolecules of aceclofenac. CarbohydrPolym. 2014Nov 26;113:138-48. doi: 10.1016/j.carbpol.2014.06.061. Epub 2014 Jul 1. PMID: 25256468.

[22] Poonuru RR, Gonugunta CS. Bimodal gastroretentive drug delivery systems of lamotrigine: formulation and evaluation. Indian J Pharm Sci. 2014 Nov;76(6):476-82. PMID: 25593380; PMCID: PMC4293678.

[23] Mohan A, Gundamaraju R. In-vitro and in vivo evaluation of fast-dissolving tablets containing solid dispersion of lamotrigine. Int J Pharm Investig. 2015 Jan;5(1):57-64. doi: 10.4103/2230-973X.147235. PMID: 25599034, PMCID: PMC4286836.

[24] Agarawal S, Jour, Sharma D et al, Formulation and evaluation of *in situ*mucoadhesivethermoreversible nasal gel of sertraline hydrochloride. Asian Journal of Pharmaceutical and Clinical Research.2019 Sep;12(11):195-202.

[25] Paul A, Fathima KM, Nair SC. Intra Nasal *In situ* Gelling System of Lamotrigine Using Ion Activated Mucoadhesive Polymer. Open Med Chem J. 2017 Dec 29;11:222-244. doi: 10.2174/1874104501711010222. PMID: 29399211; PMCID: PMC5769127.

[26] Pappas CS, Tarantilis PA, Harizanis PC, Polissiou MG. New method for pollen identification by FT-IR spectroscopy. ApplSpectrosc. 2003 Jan;57(1):23-7. doi: 10.1366/000370203321165160.PMID: 14610932.

[27] Shelke S, Shahi S, Jalalpure S, Dhamecha D, Shengule S.Formulation and evaluation of thermoreversiblemucoadhesive in situ gelfor intranasal delivery of Naratriptan hydrochloride. Journal of Drug Delivery Science and Technology.2015 Oct 1;29:238-244.

[28] Salunke SR, Patil SB. Ion activated in situ gel of gellan gum containing salbutamol sulphate for nasal administration. Int J BiolMacromol. 2016 Jun;87:41-7. doi: 10.1016/j.ijbiomac.2016.02.044.PMID: 26899173.

[29] Cai Z, Song X, Sun F, Yang Z, Hou S, Liu Z. Formulation and evaluation of in situ gelling systems for intranasal administration of gastrodin. AAPS PharmSciTech. 2011 Dec;12(4):1102-9. doi: 10.1208/s12249-011-9678-y. PMID: 21879392; PMCID: PMC3225523.

[30] Robinson JR, Eriksen SP. Theoretical formulation of sustained-release dosage forms. J Pharm Sci. 1966 Nov;55(11):1254-63. doi: 10.1002/jps.2600551118. PMID: 5969782.

[31] Rathod H, Patel V, Modasiya M. *In Situ*gel: Development, Evaluation and Optimization Using 3^2 Factorial Design. J. Pharm. Sci. & Res. 2011 Apr 1;3(4):1156-1162

[32] Cao SL, Ren XW, Zhang QZ, Chen E, Xu F, Chen J, Liu LC, Jiang XG. In situ gel based on gellan gum as new carrier for nasal administration of mometasonefuroate. Int J Pharm. 2009 Jan 5;365(1-2):109-15. doi: 10.1016/j.ijpharm.2008.08.042. PMID: 18822361.

[33] Raval, M, Bagada H. Formulation and Evaluation of Cyclodextrin-Based Thermosensitive *In Situ* Gel of Azithromycin for Periodontal Delivery. J Pharm Innov 2021 Mar;16:67-84.

[34] Mahajan HS, Gattani S. *In situ* gels of Metoclopramide Hydrochloride for intranasal delivery: in-vitro evaluation and in vivo pharmacokinetic study in rabbits. Drug Deliv. 2010 Jan 1;17(1):19-27.doi: 10.3109/10717540903447194. PMID: 19958151.

[35] Hosny KM, Banjar ZM. The formulation of a nasal nanoemulsionzaleplon in situ gel for the treatment ofinsomnia. Expert Opin Drug Deliv. 2013 Aug 1;10(8):1033-41. doi: 10.1517/17425247.2013.812069. PMID: 23795561.

[36] Swain GP, Patel S, Gandhi J, Shah P. Development of Moxifloxacin Hydrochloride loaded *in-situ* gel for the treatment of periodontitis: *In-vitro* drug release study and antibacterial activity. J Oral BiolCraniofacRes. 2019 Jul 1;9(3):190-200. doi: 10.1016/j.jobcr.2019.04.001. PMID: 31080718; PMCID: PMC6503209.

[37] Mahajan HS, Tyagi V, Lohiya G, Nerkar P. Thermally reversible xyloglucan gels as vehicles for nasal drug delivery. Drug Deliv. 2012 Jun 1;19(5):270-6. doi: 10.3109/10717544.2012.704095. PMID: 22823894.

[38] Garala K, Joshi P, Shah M, Ramkishan A, Patel J. Formulation and evaluation of periodontal in situ gel. IntJ Pharm Investig. 2013 Jan;3(1):29-41. doi: 10.4103/2230-973X.108961. PMID: 23799203; PMCID: PMC3687234.

[39] Harish NM, Prabhu P, Charyulu RN, Gulzar MA, Subrahmanyam EV. Formulation and Evaluation of in situ Gels Containing Clotrimazole for Oral Candidiasis. Indian J. Pharm. Sci. 2009 Jul;71(4):421-7. doi: 10.4103/0250-474X.57291. PMID: 20502548; PMCID: PMC2865814.

[40] Naik A, Nair H. Formulation and evaluation of thermosensitivebiogels for nose to brain delivery of doxepin. Biomed Res Int. 2014 Oct;2014:847547. doi: 10.1155/2014/847547. Epub 2014 Jun 18. PMID: 25032225; PMCID: PMC4086251.

[41] Sherafudeen SP, Vasantha PV. Development and evaluation of in situ nasal gel formulations of loratadine. Res Pharm Sci. 2015 Nov;10(6):466-76. PMID: 26779266; PMCID: PMC4698857.