Formulation and Pharmacodynamic Investigations of Lamotrigine Microspheres in Pentylenetetrazole-Induced Seizures in Mice

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Abstract

Aim: Due to the presence of rich blood circulation and non-invasive nature of administration, the nasal route has been established as valuable therapeutic alternatives. This study was designed with the basic objective to formulate mucoadhesive microspheres of anticonvulsant drug, lamotrigine (LT) for intranasal delivery and to carry out its pharmacodynamic investigations to benefit the emergency cases of epileptic seizures. **Materials and Methods:** The microspheres were prepared by ionotropic gelation method and characterized for morphology scanning electron microscopy, particle size, drug entrapment efficiency, thermal behavior (differential scanning calorimetry), and crystallinity (X-ray diffraction), *in vitro* swelling studies, *in vitro* drug diffusion, *ex vivo* bioadhesion, and *ex vivo* biocompatibility studies in excised sheep nasal mucosa. The resultant LT loaded chitosan (CH) microspheres were further evaluated for pharmacodynamic efficacy in pentylenetetrazol (PTZ) induced seizures in mice. **Result and Discussion:** Results demonstrated that the microspheres were discrete, smooth, and spherical in shape with size 24.5 ± 2.62 to 48.52 ± 2.34 µm, appropriate for nasal drug delivery. The CH-based nasal LT microspheres demonstrated high encapsulation efficacy, strong bioadhesion potential, and high permeation without any signs of morphological toxicity in excised sheep nasal mucosa. Importantly the intranasal administration of LT microspheres delayed the onset of clonic convulsion and offered complete protection against the PTZ induced seizures in mice compared to its peripheral administration. **Conclusion:** Thus, the formulation of LT loaded CH mucoadhesive microspheres offers promising advantages over conventional dosage with its immediate onset of action.

Key words: Epilepsy, ionotropic gelation method, lamotrigine, mucoadhesive microsphere

INTRODUCTION

Epilepsy is a chronic and complicated neurological disorder characterized by recurrent seizure and requires immediate rescue medication to prevent its progression to status epilepticus.[1] However, most of the antiepileptic drugs are poorly water soluble and show delayed onset of action following its oral administration. Moreover, parenteral administration is not possible as requires medical assistance. Importantly, the nasal route has gained attention for systemic drug delivery due to its great potential utility for drug delivery.

It is widely accepted that intranasal delivery of drugs provides rapid absorption to achieve effective blood level. It also provides convenience, safety as a non-invasive route and gives faster onset of action as compared to oral and transdermal route.[2] The nasal cavity offers several advantages for efficient drug delivery including relatively large surface area, porous endothelial basement membrane, highly vascularized epithelial layer, high total blood flow, and

avoids the first pass metabolism.[3] However, nasal mucociliary clearance is one of the most important limiting factors as it restricts the time of drug absorption and prevents sustained nasal drug administration. Hence, several laboratories have prepared mucoadhesive preparations to increase the contact time between the dosage form and mucosal layers of nasal cavities resulting in improved drug absorption.[4,5]

The mucoadhesive microspheres form a gel-like layer, which is cleared slowly from the nasal cavity, resulting in a prolonged residence time of the drug formulation.[6-8] Specifically mucoadhesive microspheres of antiepileptic drugs can provide more contact time and enhance the absorption of the drug to produce sufficient therapeutic level.[9] Lamotrigine (LT) is an antiepileptic drug approved for the treatment of epilepsy and bipolar disorder. In particular, it is used to treat partial seizures and tonic-clonic convulsions.[10] It also acts as a mood stabilizer. Chemically unrelated to other anticonvulsants, it has relatively few side-effects and does not require therapeutic drug monitoring. In view of the potential pharmacological application of LT in the treatment of epilepsy, the present work was designed to formulate its mucoadhesive microspheres using ionotropic gelation method and to carry out its pharmaceutical evaluation.

A number of biodegradable materials have been traditionally used as carriers for microparticulate drug delivery systems. In recent times, chitosan (CH) microspheres have received substantial attention owing to its biodegradable, biocompatible, mucoadhesive, and nontoxic nature. Further, the gelling characteristics of CH provide additional advantages for microencapsulation of drugs via microparticulate systems.[11] Hence, CH was selected as carrier for microspheres.

In this study, CH microspheres were prepared by ionotropic gelation method. The influence of various process and formulation parameters, like CH concentration and volume of crosslinking agent on the particle size of CH microspheres cross-linked with glutaraldehyde was investigated.

MATERIALS AND METHODS

Materials

LT was received as a gift sample from Glenmark, Mumbai. CH (DD: 75-85%) was obtained from Himedia Laboratories Pvt. Ltd., Nashik, while glutaraldehyde was purchased from Rankem, Nagpur. All the chemicals used in experiments were of analytical grade.

Methods

Preparation of LT microspheres

As shown in Table 1, total eight batches of LT microspheres were prepared by ionotropic gelation method using various

concentration of drug-polymer CH and crosslinking agent glutaraldehyde. Briefly, CH was dissolved in 1% v/v aqueous acetic acid solution to obtain its cationic solution. LT was dissolved in CH cationic solution. The polyanionic glutaraldehyde solution was then added to the above resultant solution in drop-wise manner at the rate of 30 drops per min from 5 cm height with constant stirring at 800 rpm using mechanical stirrer. Microspheres obtained herein were collected by filtration, washed with deionized water and dried in oven up to the temperature of 60°C for 3 h. Blank microspheres were also prepared using the same procedure as described earlier without drug.[12] For optimization of LT microspheres, the varied concentration of drug, polymer and crosslinking agent were used.

Physicochemical and morphological characterization

The resultant microspheres were critically analyzed for physicochemical parameters such as particle size, polydispersity index (PDI), and zeta potential. All the batches were analyzed for particle size using digital Motic microscope (model no: B1-223SP). Surface morphology was determined by scanning electron microscopy (SEM) (JEOL Model JSM - 6390LV). Samples for SEM study were prepared by lightly sprinkling the formulation on a double-adhesive tape stuck to an aluminum stub. The stubs were then coated with gold to a thickness ~300 A° under an argon atmosphere using a gold sputter module in a high vacuum evaporator. The coated samples were then randomly scanned, and photomicrographs were taken with SEM.[13] PDI and zeta potential of microspheres were determined by dynamic light scattering using zetasizer (Nano ZS90, Malvern Instruments Ltd., Malvern, UK).

Fourier transform infrared (FTIR) spectroscopy

The spectra were recorded in solid state for LT, CH, and LT loaded CH microspheres using FTIR (Thermo Nicolet, Avatar 370). The samples were prepared as potassium bromide (KBr) disks on an FTIR spectrophotometer (2 mg sample in 200 mg KBr ratio) and scanned in a range 400-4000/cm as described earlier.[14]

Differential scanning calorimetry (DSC)

The thermal profile of LT, CH, and LT loaded CH microspheres was recorded on DSC (Mettler Toledo DSC 822e). The thermograms were obtained by heating the microspheres at rate of 10°C/min from 30°C to 300°C using nitrogen purge of 50 ml/min.

X-ray diffraction studies

The X-ray diffractograms of LT, CH, and LT loaded CH microspheres were recorded on an X-ray diffractometer (Brucker AXS D8) to evaluate its crystallinities. Diffractograms were scanned in the range from 3°C to 80°C (2θ) with resolution of 0.02°C and scanning speed of 2.0°C/min. An accelerating voltage of 40 kV was applied at the current intensity of 35 mA.

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Table 1: Composition of different batches of lamotrigine loaded chitosan microspheres								
Lamotrigine (mg)	100	100	100	100	200	300	100	100
Chitosan (mg)	100	200	300	400	100	100	200	200
Glutaraldehyde (ml)	2	2	2	2	2	2	3	5

Percentage yield

The dried microspheres were weighed and percentage yield was calculated with respect to the initial amount of LT and CH used for the preparation of microspheres.

Encapsulation efficiency

The encapsulation efficiency was determined by earlier methods with slight modifications.[13] Briefly, microspheres containing 10 mg LT were dissolved in methanol and kept overnight to extract the drug. The samples were centrifuged at 560 rpm for 10 min to eliminate the nonsoluble residue. The resultant solution was filtered and the filtrate was analyzed for the drug content by ultraviolet (UV)-visible spectrophotometer at 305 nm (Shimadzu 7800, Tokyo Japan). Methanol was used as blank. The data were collected by repeating the procedure in triplicate. Encapsulation efficiency was determined by the following equation:

$$E = Q_p/Q_t \times 100$$

Where, E = Percent drug encapsulation in microspheres, Q_t = Quantity of the drug added and Q_p = Quantity of drug encapsulated in microspheres.

In vitro bio-adhesion

The *in vitro* bioadhesion studies of microspheres were performed by falling liquid film technique as described earlier.[15] Briefly, fresh sheep nasal mucosa was obtained from local slaughter house (Kamptee), thoroughly extracted, washed with saline solution and mounted on a glass slide. Weighed quantity of (50 mg) microspheres was carefully sprinkled on mucosa. Thereafter, 100 µL of simulated nasal electrolyte solution (SNES: Containing 8.77 mg/ml NaCl, 2.98 mg/ml KCl and 0.59 mg/ml $CaCl_2$ per liter) was spread on the microspheres and incubated for 15 min in a desiccator at 90% relative humidity. Pre-warmed phosphate buffer (pH 6.6) was peristaltically pumped at the rate of 5 ml/min over the sheep nasal mucosa. After 1 h, the concentration of LT in the collected perfusate was determined at the wavelength of 305 nm using UV-visible spectrophotometer. The amount of microspheres equivalent to the amount of drug in the perfusate was determined. The mucoadhesion potential was determined by the following equation:

Mucoadhesion potential (%) = (Concentration of adhered MS)/(Concentration of applied MS) ×100.

In vitro swelling studies

The swelling ability of prepared microspheres in physiological media was determined by allowing them to swell to their equilibrium.

Accurately weighed amounts of microspheres (10 mg) were placed on millipore filter (NY 11 0.22 mm) using a Franz diffusion cell (12 ml capacity) with phosphate buffer (pH 6.6). The microspheres were periodically removed, blotted with filter paper and their changes in weights were measured during the swelling until equilibrium was attained. Finally, the weight of the swollen microspheres was recorded after a period of 3 h, and the swelling index was then calculated from the following formula.[13] The studies were carried out in triplicate.

$$\text{Swelling index} = \frac{W_e - W_o}{W_o} \times 100$$

Where,
W_o = Initial weight of the dry microspheres,
W_e = Weight of the swollen microspheres at equilibrium.

Ex vivo biocompatibility studies

The histopathological studies were performed to ensure the biocompatibility of microspheres with sheep nasal mucosa. The freshly excised nasal mucosa of sheep was collected and cleaned with saline solution. After application of predefined amounts of LT microspheres (100 mg), mucosal tissues were fixed in 10% formalin solution and implanted in paraffin. Paraffin sections (7.5 µm) were stained with Hematoxylin and Eosin (H and E) and observed under digital Motic microscope (Model no B1-223SP). The untreated mucosa incubated with phosphate buffer solution (pH 6.6) was used as a control.[16]

In vitro drug diffusion studies

The *in vitro* drug diffusion test of microspheres was performed using Franz diffusion cell across dialysis membrane (Av diameter 21.5 mm, Av flat width 32.34 mm) as diffusion barrier. The membrane was equilibrated overnight with pH 6.6 phosphate buffer before dispersing the microspheres into the supplier compartment. The receptor compartment was filled with phosphate buffer solution (pH 6.6) that was within the pH range in the nasal cavity. The supplier compartment was placed in such a way that it just touched the diffusion medium in the receptor compartment. The temperature was maintained at 37°C ± 1°C using circulating water bath. 300 µl

of sample was withdrawn at predetermined time points from the receptor compartment, replaced with the same amount of fresh pre-warmed buffer solution and assayed for LT using UV-visible spectrophotometer at 305 nm.

Ex vivo permeation studies

Ex vivo drug permeation studies of microspheres were carried out using Franz's diffusion cell (receptor capacity: 17.0 ml; permeation area 3.14 cm^2). Again sheep nasal mucosa was used as prototypical membrane. Permeation medium phosphate buffer (pH 6.6, 37°C ± 1°C) was filled in the receptor compartment with constant stirring. The nasal mucosa was placed on the diffusion chamber with mucosal and serosal surfaces directed toward supplier and receptor compartments, respectively. Microspheres equivalent to 10 mg of LT were spread over the mucosal membrane in supplier compartment previously soaked with 3.5 ml SNES. 300 µl of medium from receptor compartment was withdrawn at predetermined time points (total 9 withdrawals at 10 min interval). The withdrawal amount was replaced by fresh phosphate buffer solution (pH 6.6) and analyzed by UV-visible spectrophotometer at 305 nm.[17]

Pharmacodynamic studies

Subjects

Adult male Swiss Albino mice (National Institute of Nutrition [NIN], Hyderabad, India) weighing 22-25 g were housed in standard laboratory conditions of temperature (23°C ± 1°C) and relative humidity (55% ± 5%), with free access to food (NIN, Hyderabad, India) and water. Animals were divided into three groups (*n* = 6), fasted overnight before the experiments and transferred to the laboratory at least 1 h before the beginning of the experiment. The experiments were performed during light cycle in between 9.00 and 13.00 h. For intranasal administration, animals were held in a supine position under light ketamine (100 mg/kg)/xylazine (10 mg/kg) anesthesia. Intranasal doses (equivalent to LT: 5 mg/kg, divided evenly between both nostrils) of microsphere suspension were administered using a polyethylene tube attached to Hamilton syringe. The tube was inserted about 5-6 mm deep into each nostril for the proper delivery of the drug into the nasal cavity. In separate groups, animals were injected with suspension of LT loaded CH microsphere (equivalent to 5 mg/kg of LT) via intraperitoneal (IP) route. 30 min following drug administration, animals were subcutaneously injected with pentylenetetrazol (PTZ) (80 mg/kg), and the onset of clonic convulsions and percentage protection in each group were recorded.[18]

Statistical analysis

All the results are reported as mean ± standard deviation (SD) or mean ± standard error mean (SEM). Statistical comparisons were performed by one-way analysis of variance followed by Dennett's multiple comparison tests. Differences between formulations were considered to be statistically significant at $P < 0.05$ in all cases.

RESULTS AND DISCUSSION

Due to the presence of rich blood circulation and non-invasive nature of administration, the nasal route has been established as valuable alternatives in the treatment of several disorders. This study emphasized the importance of nasal delivery of anticonvulsant drug, LT via mucoadhesive microspheres for emergency conditions of epileptic seizures.

Preparation and characterization of microspheres

Preformulation studies were performed for drug solubility, CH solubility and method of preparation for the development of mucoadhesive microspheres. It is important to mention here that initially microspheres were prepared by emulsification crosslinking method and ionic gelation method. However, particle size of microspheres obtained by emulsification crosslinking method was >56.7 ± 1.43 µm, whereas by ionic gelation method it was <48.5 ± 2.34 µm. This resultant particle size (<50 µm) is suitable for deposition in the nasal cavity without the risk of passing to the lower respiratory tract.[19-21] Hence, ionotropic gelation method was further selected for the preparation of microspheres using CH as carrier polymer and glutaraldehyde as crosslinking agent. It is simple process produces complete hydrophilic environment of CH particles by avoiding the use of organic solvents[22] and or surfactants commonly used in emulsification and solvent evaporation method.[23,24] Glutaraldehyde has been commonly used as a cross-linking agent owing to its economy, availability and highly solubility in aqueous solution.[25] The high reactivity of the aldehyde groups, which readily form imine bonds (Schiff's base) with amino groups and acetal bonds with hydroxyl groups,[26] provides the efficiency of glutaraldehyde on the cross-linking of CH.

Physicochemical characterization of microspheres

The bioavailability of the drug by nasal route mainly governs by physicochemical property and mucociliary clearance. In this context, CH microspheres offer specific advantages, as it reduces time for mucociliary clearance and prolongs contact time with nasal mucosa for better drug administration. The particle size of different microspheres is shown in Table 2. The particle size of microspheres varies with the concentration of CH used. At 1% CH concentration, the particle size of microspheres was 24.5 ± 2.62 µm which increase to 48.5 ± 2.34 µm at 3% of CH concentration. This resultant particle is suitable for deposition in the nasal cavity without the risk of passing to the lower respiratory tract.[19-21] The zeta potential of prepared microspheres as determined by zetasizer was found to increase from 28.3 to 51.4 mV for

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

Table 2: Characterization of lamotrigine loaded chitosan microspheres

*Values expressed as mean±SD, *n*=3, #indicates average of 100 particles±SD. SD: Standard deviation

the increase in ratio of LT to CH from 1:1 to 1:4, respectively, in accordance with the earlier findings.[27] In addition, the PDI was found to be as low as 0.473 indicating a narrow range of particle size distribution. As reported by earlier findings an optimum diameter for nasal microspheres has to be >5-10 µm.[28-32] Particles <5 µm escape to the lungs, whereas larger particles may deposit on the nasal mucus membrane, with larger ones depositing more anteriorly.[33,34] It is important to note that, in our study, particle size of the microsphere was directly proportional to the concentration of CH used and at high concentration clusters of microspheres were observed. This may be because of increased viscosity of the CH solution that may give larger particles with aggregation.

Morphology

Morphology of prepared microspheres was determined by SEM. LT loaded CH microspheres showed the regular shape and smooth surface [Figure 1]. There was negligible presence of free drug sample. This indicates that LT was completely loaded into polymeric network prepared by inotropic gelation method. The resultant microspheres did not show any ruptures on surface validating its rapid clearing from the nasal cavity with optimum permeation.

FTIR spectroscopy

FTIR spectra of LT, CH, and LT loaded CH microspheres are shown in Figure 2. The spectrum of LT is characterized by the presence of strong absorption band at 3451/cm, 3318/cm and 3267/cm, which are all indicative of amines (-NH-group). The carbonyl stretching mode appears as a very strong doublet at 1600/cm (C=O stretching) and at 800/cm, which was indicative of the presence of aromatic rings. Characteristic peak of CH was observed at 3443/cm for the OH group, 1654/cm for carbonyl stretching vibration, 2880/cm for C-H bonds, and 1378/cm for the amino groups. The careful observation of the FTIR spectra of LT loaded microspheres revealed that the major FTIR absorption peaks

viewed in the spectra of the drug were close to those in the spectra of the microspheres. It indicates that the method of preparation and processing parameters has not affected the drug stability.

DSC

DSC investigates the thermal properties of the prepared microspheres, providing qualitative information about the physicochemical state of the drug within the microspheres. DSC thermograms of LT, CH, and LT loaded CH microspheres are shown in Figure 3. DSC thermogram of LT showed a sharp endothermic peak at 218.29°C indicating melting point of LT. Thermogram of LT loaded CH microspheres revealed the partial absence of endothermic peak. Thus, it is possible that drug might have been dispersed in crystalline or amorphous form or dissolved in the polymer matrix during formation of the microspheres. In fact, it is observed that if there is no noticeable endotherm, the drug might be present in a molecular dispersion or solid solution state in the polymeric microspheres loaded with the drug.[35] This could also attribute to the incorporation and molecular dispersion of LT in CH polymer matrices of the prepared microspheres formulations.

X-ray diffraction studies

To characterize the physical state of LT loaded CH microsphere X-ray diffraction studies were carried out. The X-ray diffraction patterns of pure LT, CH, and LT loaded CH microspheres are shown in Figure 4. The distinctive sharp peaks were observed at various diffraction angles on 2θ scale; demonstrate the typical crystalline nature of LT. However, X-ray diffraction pattern of LT loaded CH microspheres did not show any peaks suggesting that the LT might have been molecularly dispersed in the CH or converted in amorphous form in CH microspheres.[36] The results are also in accordance with the DSC analysis of LT CH microsphere.
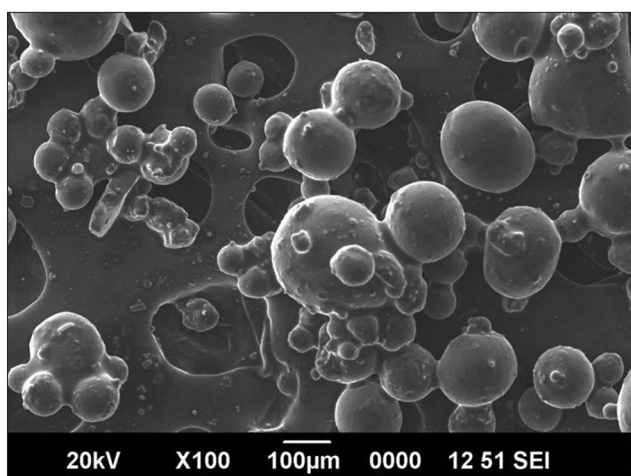
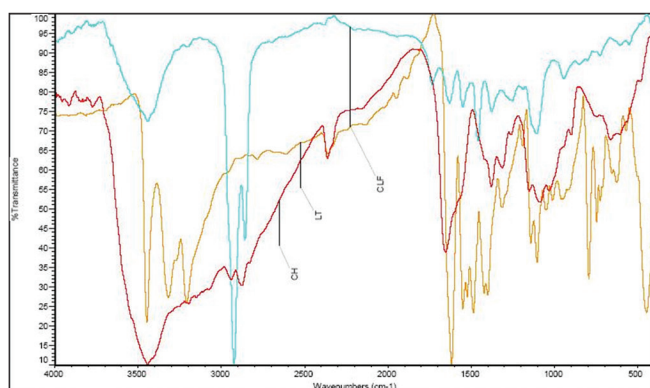**Figure 1:** Scanning electron microscopy image of lamotrigine loaded chitosan microspheres

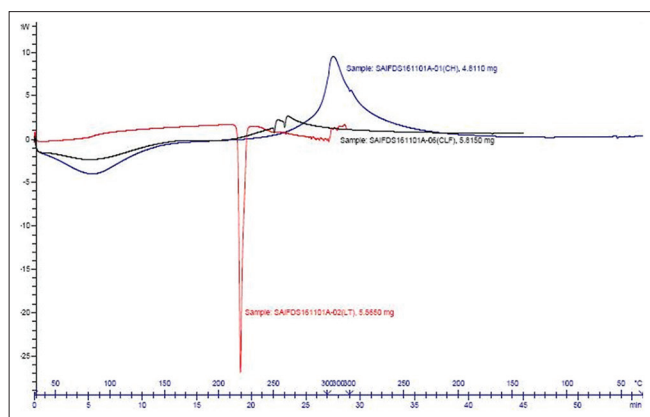**Figure 2:** Fourier transform infrared spectra of lamotrigine, chitosan and lamotrigine loaded chitosan microspheres

Figure 3: Differential scanning calorimetry thermograms of lamotrigine, chitosan and lamotrigine loaded microspheres

Percentage yield

The percentage yield was calculated to determine the yield of microspheres obtained by the ionotropic gelation method. All the batches showed percentage yield ranging from 65% to 95%.

Encapsulation efficiency

The resultant LT loaded microspheres showed relatively high drug encapsulation efficiency. As shown in Table 2, the encapsulation efficiency was amplified as the LT: CH ratio increased. In general, microspheres prepared by ionotropic gelation technique exhibited significantly higher drug encapsulation efficiency. This is supported by earlier studies employing CH microspheres by ionotropic gelation technique.[37] It is noteworthy that F1 and F2 batches prepared at LT: CH ratio (1:1) and (1:2) showed the highest entrapment efficiency as 86.15 and 84.91%, respectively. Thus, we have considered these microsphere formulae (F1 and F2) for further characterization and evaluation.

Ex vivo bioadhesion study

The *ex vivo* bioadhesion studies were carried out to determine the adhesion of formulations to nasal mucosa so as to prevent the removal of the drug from the site and also for enhanced permeation of drug by nasal route. The prepared microspheres showed good bioadhesion strength ranging from 60.54% ± 1.48% to 85.75% ± 1.83% as studied on excised sheep nasal mucosa. As shown in Table 2, CH concentration showed significant influence on bioadhesive capability of microsphere using ionizing gelation method. It is worth mentioning that, we have used CH polymer having high molecular weight (310-375 kDa) which exhibits strong mucoadhesive property as compared to low molecular weight CH.[38] Further, it is widely accepted that the particle with surface charge density like CH can serve as good mucoadhesive agents. The interaction between cationic group on CH and anionic residues such as sialic acid and sulfonic acid on the nasal mucosa might have facilitated the strong adhesion of LT loaded CH microsphere on mucosal surfaces. This is speculated by several earlier findings employing CH as polymer for the preparation of microsphere for nasal drug delivery system.

In vitro swelling studies

The CH ratio in microspheres had a direct effect on the swelling ability in phosphate buffer (pH 6.6). As the ratio of CH to drug increased, the swelling index increased significantly ($P < 0.05$), possibly due to increased number of the positively charged amino groups, hence capturing less water. The % equilibrium water uptake of the microspheres was ranged from 82.77% ± 2.28% to 124.23% ± 2.99% as illustrated in Table 2. As the amount of glutaraldehyde was increased, the equilibrium water uptake was decreased. This might be due to the formation of a rigid network structure at higher concentration of crosslinking. Hence, the crosslinking of microspheres has a great influence on the equilibrium water uptake.
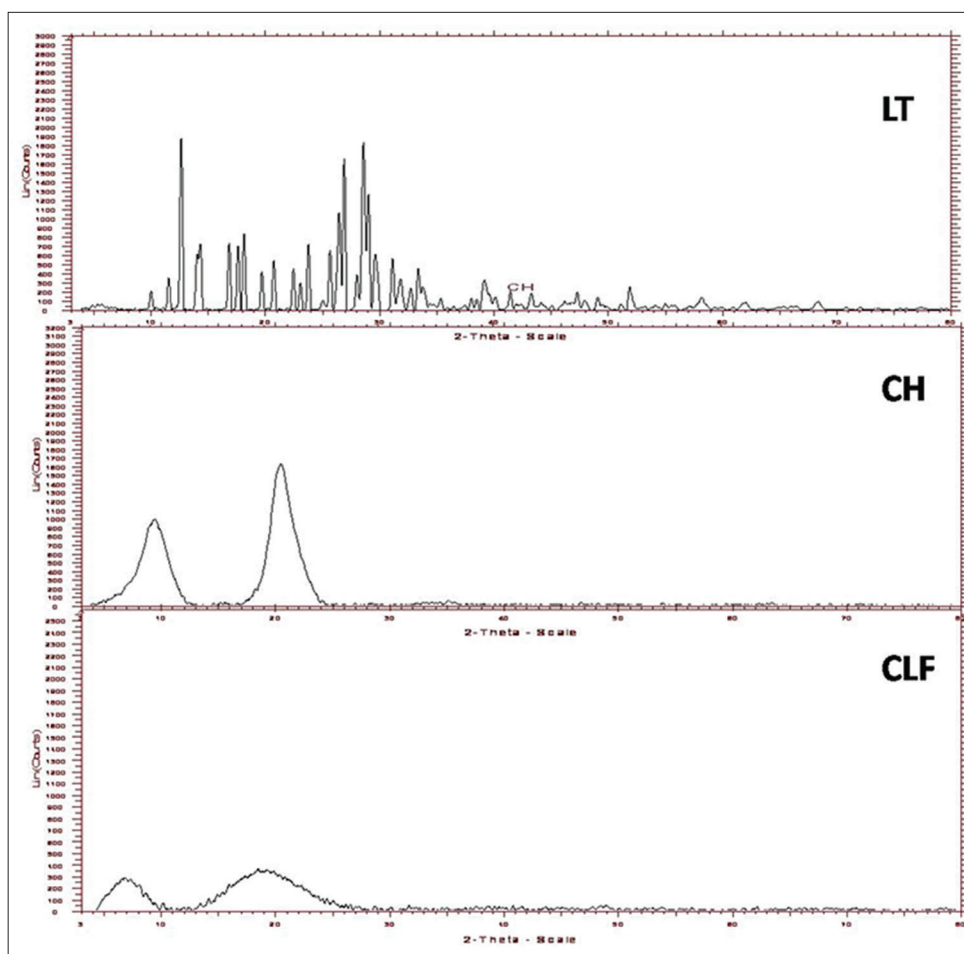
Figure 4: X-ray diffraction patterns of lamotrigine, chitosan and lamotrigine loaded microspheres

Ex vivo biocompatibility studies

The safety issues over the development of nasal mucoadhesive microspheres have been highly disputed. Thus, it is important to maintain the nasal mucosal integrity during preparation of nasal microspheres. Figure 5 illustrates the histopathological specimen of untreated nasal mucosa and treated with LT loaded CH microspheres nasal mucosa stained with H and E. Tissue examination showing ciliated nasal epithelium and goblet cells in untreated nasal mucosa. In treated mucosa, the epithelial cells appeared as intact layers. No significant changes or sign of damage was observed on the treated mucosa. There were no apparent signs of any epithelial necrosis or sloughing of epithelial cells on the microsphere-treated nasal mucosa. The biocompatibility of LT loaded CH microspheres is consistent with earlier reports that showed that CH is the least toxic polymer owing to its high degree of deacetylation and can be applied on the nasal epithelium.[39,40]

In vitro drug release study

The release of LT from microspheres was immediate and continued up to 90 min. For all the prepared microspheres, release rate of LT was inversely proportional to drug/polymer ratio; the lower the ratio the faster the release. The release from F1 and F2 microspheres was relatively high compared to other batches (i.e., F3-F8) [Figure 6]. This is particularly important for prompt absorption of LT to reach the desired drug concentration in plasma after nasal administration could be beneficial in the emergency cases of epileptic attacks. Other studies on microspheres incorporating range of poorly water insoluble drugs through nasal route have demonstrated dissolution enhancement when entrapped in CH matrix including prednisolone,[41] carbamazepine,[22] and dexamethasone.[42] The improvement of the dissolution of the drugs from the microspheres was attributed to their small size and entrapment efficiency that may lead to the uniform dispersion of the drug into the polymeric network.[43]

Ex vivo permeation studies

The *ex vivo* permeation studies was performed for the optimized formulation F1 and F2 (drug: polymer ratio is 1:1 and 1:2) due to its favorable particle size (25 μm), high entrapment efficiency (above 80%) as well as fast release rate (within 90 min). The permeation (drug release) of LT from microspheres of batch F1 and F2 was found to be in the range of 86.29% and 81.87%, respectively, as depicted in
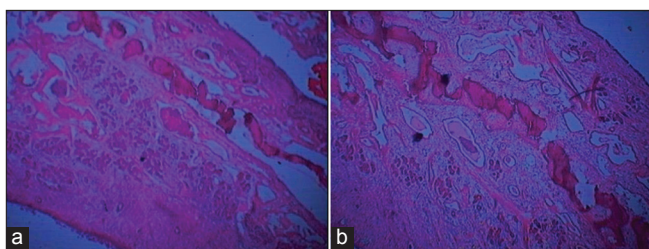
Figure 5: Light photomicrograph of sheep nasal mucosa, untreated control mucosa (a) and lamotrigine loaded chitosan microspheres-treated mucosa (b)
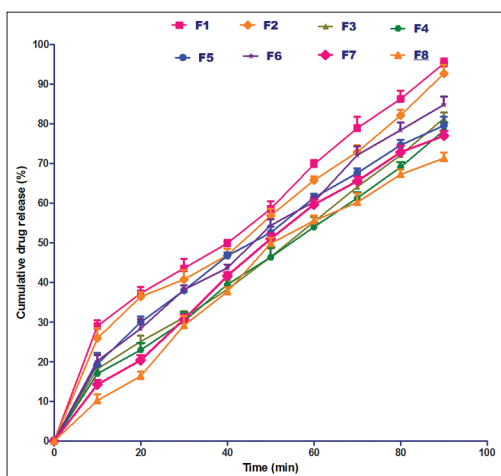

Figure 6: *In vitro* drug release profiles of lamotrigine loaded chitosan microspheres

Figure 7. CH is a polymeric material can be used to increase the drug dissolution and absorption through microspheres formulations of poorly water soluble drugs.[22] Smaller particle size of microspheres provides a larger surface area that increases the drug release from the formulations.

Pharmacodynamic studies

As showed in Figure 8, administration of PTZ to vehicle-treated mice produced clonic convulsions in all animals, and the onset of such convulsions was 78.83 ± 6.4 s. Intranasal administration of LT loaded CH microspheres significantly delayed the onset clonic seizures in PTZ injected animals as compared to its peripheral administration. Importantly, intranasal LT loaded CH microsphere offered 100% protection against the mortality induced by PTZ which was 33.33% in IP LT injection. The results clearly indicate the greater anticonvulsant effect of LT in PTZ induced seizures if given by intranasal route compared to its peripheral administration. The results of our study are well supported by recent finding that intranasal administration of LT achieved high bioavailability in different brain areas involved in the pathogenesis of epilepsy.[44] Thus, intranasal administration of LT via mucoadhesive microspheres may be appropriate and valuable drug delivery system for the chronic and acute attacks of epileptic seizures.
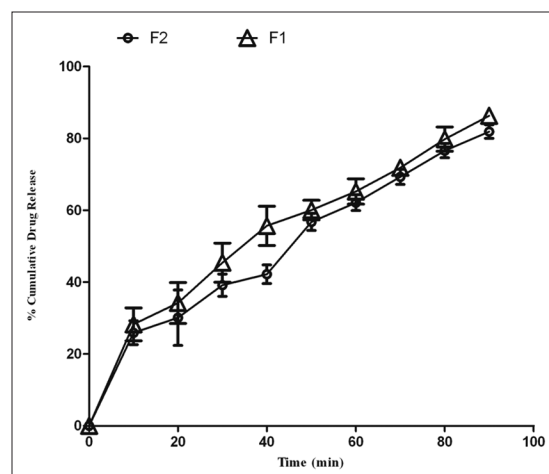

Figure 7: *Ex vivo* lamotrigine permeation through nasal mucosa from mucoadhesive microspheres
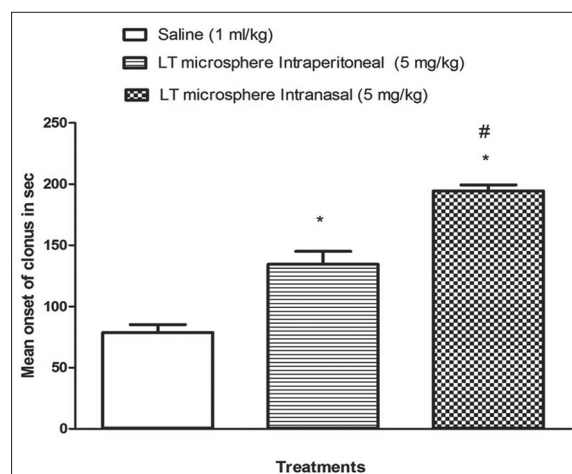

Figure 8: Effect of lamotrigine microspheres on mean onset time of convulsions against pentylenetetrazol-induced seizures. *$P < 0.0001$ as compared to control group; #$P < 0.0001$ as compared to intraperitoneal injected group (ANOVA followed by Dunnett's test) ($n = 6$)

CONCLUSION

In this study, we have prepared and optimized LT loaded CH mucoadhesive microspheres for nasal administration employing ionic gelation method. Physicochemical investigations demonstrated that the LT microspheres showed suitable particle size for nasal administration, high encapsulation efficacy, and strong bioadhesion potential without any morphological toxicity to excised sheep nasal mucosa. In addition, permeation, across excised sheep nasal mucosa exhibited good permeability of LT loaded CH microspheres. Importantly the intranasal administration of LT microspheres delayed the onset of convulsion and offered the complete protection against the PTZ induced seizures compared to its peripheral administration. Thus, the formulation of LT loaded CH mucoadhesive microspheres offers promising advantages over conventional dosage with

its immediate onset of action. It would be beneficial to carry out *in vivo* pharmacokinetic profile and clinical studies of LT loaded CH microspheres to project its therapeutic efficacy in emergency cases of epileptic seizures.

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