INTRODUCTION
Pregabalin an anticonvulsant agent binds with high affinity to the α₂ - δ (α₂–δ) auxiliary protein of voltage-gated calcium channels. It prevents both partial seizures and generalized seizures in humans. Although, pregabalin shows high oral absorption and greater degree of intrinsic efficacy to prevent seizure activity, its delayed transport across the blood-brain barrier limits the use in emergency epileptic seizures. Due to the particular anatomical features of the nasal cavity, intranasal (IN) administration has been explored as a means of preferential drug delivery to the brain. The objective of the present study was to improve therapeutic efficacy through IN administration of pregabalin-loaded mucoadhesive microspheres. Materials and Methods: Chitosan-based mucoadhesive microspheres were prepared by ionotropic gelation method using glutaraldehyde as a crosslinking agent. Effect of chitosan concentration, glutaraldehyde concentration, and the stirring speed was investigated for the formation of microspheres. The physicochemical properties of microspheres, including particle size, zeta potential, drug content, entrapment efficiency, swelling index, and morphology were examined using suitable tools. Ex vivo bioadhesion, biocompatibility, and drug permeation were determined using sheep nasal mucosa. Anticonvulsant effect of pregabalin-loaded microspheres was investigated in pentylenetetrazole (PTZ) induced seizures in mice through IN administration. Results: Pregabalin-loaded chitosan microspheres were spherical shape 18.2–24.35 µm mean diameter, positive zeta potential, 80% entrapment efficiency, and 87.29% ex vivo drug permeation. The results of in vivo studies clearly indicated the greater anticonvulsant effect pregabalin in PTZ-induced seizures by IN route as compared to its peripheral administration. Conclusion: Thus, IN administration of pregabalin via mucoadhesive microspheres may be appropriate and valuable drug delivery system for the chronic and acute attacks of epileptic seizures.

KEY WORDS: Chitosan, Epilepsy, Intranasal, Microspheres, Pregabalin
slowly from the nasal cavity, resulting in a prolonged residence time of the drug formulation.\[^{[5]}\] Specifically, mucoadhesive microspheres of antiepileptic drugs can provide more contact time and enhance the absorption of the drug to produce sufficient therapeutic level.\[^{[7]}\] In this investigation, our primary objective was to prepare and characterize IN microspheres of pregabalin with chitosan as bioadhesive carrier as well biodegradable, biocompatible, and nontoxic in nature. In addition, the influence of various process and formulation parameters, such as polymer concentration, stirring rate, the volume of cross-linking agent, and on the particle size of chitosan microspheres (CPF) was also investigated.

**MATERIALS AND METHODS**

**Materials**

Pregabalin was procured from Glenmark Pharmaceutical Ltd., Mumbai. Chitosan was purchased from Hi-media Laboratories, Mumbai and glutaraldehyde 25% from Rankem, Nagpur (Maharashtra). All the chemicals, reagents and solvents used were of analytical grade.

**Subjects**

Adult male Swiss Albino mice were obtained from the National Institute of Nutrition; Hyderabad, (India), weighing 22–25 g housed in standard laboratory conditions of temperature (23 ± 1°C) and relative humidity (55 ± 5%) at animal Centre for Laboratory Research, SKB College of Pharmacy, Kamptee, with free access to food and water. All the experimental protocols were approved by Institutional Animal Ethics Committee of the SKB College of Pharmacy, Kamptee and performed according to guidelines of CPCSEA.

**Methods**

**Preparation of pregabalin loaded CPF**

Mucoadhesive microspheres were prepared by ionotropic gelation method using chitosan as bioadhesive polymer and crosslinked with glutaraldehyde. Glutaraldehyde is polyanionic which interact with a positively charged amino group of chitosan by electrostatic forces. In brief, pregabalin (1% [w/v]) was dissolved in stirred solution of chitosan prepared in 1% (v/v) acetic acid solution until a uniform dispersion was obtained. The microspheres were formed by dropping the bubble free glutaraldehyde at the rate of 30 drops/min from 5 cm height through a 21G disposable syringe into above dispersion mechanically agitated (800 rpm). Due to complexation between oppositely charged species, chitosan undergoes ionic gelation, and precipitates to form spherical particles. The microspheres formed were separated after 30 min, washed with deionized water and then subsequently dried at 60°C for 3 h. Blank microspheres were prepared using same procedure as stated above excluding pregabalin.\[^{[8]}\] The composition of different formulations is shown in Table 1.

**Calibration curve of pregabalin**

A spectrophotometric method based on ninhydrin derivatization was used for the preparation of calibration curve and determination of pregabalin from microspheres.\[^{[9]}\] Stock solution 100 µg/ml of pregabalin was prepared by dissolving 10 mg of pregabalin to 100 ml of phosphate buffer (pH 6.6). A series of working solutions ranging from 2 to 14 µg/ml were prepared from standard stock solution. To each working solution, 1 ml of ninhydrin was added and incubated in the water bath of 70°C for 20 min. After cooling to the room temperature, the absorbance of the solutions was measured spectrophotometrically at 403 nm. The same procedure was used for the determination of drug content in microspheres, in vitro drug diffusion and ex vivo drug permeation.

**Characterization of microsphere**

**Surface morphology and size of microspheres**

The shape and surface morphology of microspheres were observed by scanning electron microscopy (SEM) (JEOL Model JSM - 6390LV). The samples 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 Å 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. The average particle size of the prepared microspheres was determined by Motic digital microscope (Model no B1-223SP). The particle sizes of 100 microspheres were determined randomly. The average particle size of microspheres determined using following formula:

\[
\text{Average Size} = \frac{\sum nd}{\sum n}
\]

Where, \(n\) is the number of microspheres and \(d\) is the size of microsphere.

**Polydispersity index (PDI) and zeta potential**

PDI and zeta potential of microspheres was determined by photon correlation spectroscopy using Zetasizer (Malvern Instruments Ltd., Ver. 6.2) at 25°C and at a scattering angle of 90°, maintaining electric field strength of 25 V m\(^{-1}\). In brief, weighed amount of sample (5 mg) was dispersed in 10 ml of bidistilled water to get optimum 100–200 kilo-counts s\(^{-1}\) (kcps) for measurements and then, mean diameter and PDI were determined in the range of 1 nm to 10,000 nm and zeta potential in the range of +200–−200 mv. Results were presented as mean ± standard deviation (SD) from three replicate determinations.
Determination of drug content and entrapment efficiency

The drug content and entrapment efficiency were determined in triplicate according to the reported method. Microspheres equivalent to 10 mg of drug were dissolved in 20 ml of methanol used as common solvent for drug and polymer and kept overnight to extract the drug. The samples were centrifuge at 560 rpm for 10 min to eliminate the nonsoluble residue. The resultant solution was filtered, and 1 ml of filtrate was analyzed for the drug content by ultraviolet (UV)-visible spectrophotometer (Analytical Technologies Ltd., Gujarat, India) at 403 nm. Methanol was used as a blank. Drug content was determined by the following formula:

\[
\text{Drug content} \%= \frac{Q_p}{Q} \times 100
\]

Where, \( Q_p \) = Quantity of drug encapsulated in microspheres.
\( Q \) = Weighed quantity of powder of microspheres.

The entrapment efficiency (%) of the drug was calculated using the formula:

\[
\text{Entrapment efficiency (E)} = \frac{Q_p}{Q_t} \times 100
\]

Where, \( E \) = Percentage of encapsulation of microspheres.
\( Q_p \) = Quantity of drug encapsulated in microspheres.
\( Q_t \) = Quantity of the drug added for encapsulation.

Ex vivo bioadhesion study

The ex vivo bioadhesion studies of microspheres were performed by falling liquid film technique as described earlier. Fresh sheep nasal mucosa was obtained from the local slaughterhouse, within 1 h of sacrificing the animal. A piece of 2 cm² was cut and cleaned by washing with an isotonic saline solution. An accurately weighed quantity of microspheres (100 mg) was placed on the mucosal surface, and it was attached to a polyethylene plate. About 100 ml of simulated nasal electrolyte solution (SNES: Aqueous solution containing 8.77 mg/ml NaCl, 2.98 mg/ml KCl, and 0.59 mg/ml CaCl₂/L) was put on microspheres and this plate was incubated for 15 min in desiccators at 90% relative humidity to allow the polymer to interact with the membrane. Further, the plate was fixed at an angle of 45° relative to the horizontal plane. Preheated phosphate buffer pH 6.6 at 37°C was peristaltically pumped at a rate of 5 ml/min over the tissue and the perfusate was collected in a beaker. After 1 h, the amount of drug in collected perfusate was determined spectrophotometrically at 403 nm. The amount of microspheres corresponding to the amount of drug in the perfusate was determined. The amount of adhered microspheres was calculated as the difference between the amount of applied microspheres and the amount of flowed microspheres. The percent mucoadhesion was determined by the following equation:

\[
\text{Mucoadhesion potential (\%)} = \frac{\text{concentration of adhered MS}}{\text{concentration of applied MS}} \times 100
\]

In vitro swelling studies

Accurately weighed the amount of microspheres (10 mg) were placed on millipore filter (NY 110.22 mm) using a Franz diffusion cell (17 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 at equilibrium, and the swelling ratio was then calculated from the following formula. The studies were carried out in triplicate.

\[
\text{Swelling Index} = \frac{\text{We}-\text{Wo}}{\text{Wo}} \times 100
\]

Where,
\( \text{Wo} \) = Initial weight of the dry microspheres.
\( \text{We} \) = Weight of the swollen microspheres at equilibrium swelling in the media.

Differential scanning calorimetry (DSC)

The DSC study was performed with a DSC (Mettler Toledo DSC 822e) at rate of 10°C min⁻¹ from 30° to 300°C using nitrogen purge of 50 ml/min. Pure water and indium were used as primary standard for calibration of the instrument for temperature and heat flow using the DSC temperature scale and enthalpy.

### Table 1: Composition of different batches of pregabalin-loaded chitosan microspheres

<table>
<thead>
<tr>
<th>Formulation ID</th>
<th>Pregabalin mg</th>
<th>Chitosan mg</th>
<th>Glutaraldehyde ml</th>
<th>Stirring speed</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>100</td>
<td>100</td>
<td>2</td>
<td>800</td>
</tr>
<tr>
<td>P2</td>
<td>100</td>
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<tr>
<td>P10</td>
<td>100</td>
<td>200</td>
<td>2</td>
<td>1200</td>
</tr>
</tbody>
</table>
response as standard. All the samples were weighed (8–10 mg) and encapsulated into close aluminum pans which were then subsequently crimped to ensure a tight seal and measured under a nitrogen atmosphere.[12]

X-ray diffractometry (XRD)
The crystalline state of the drug, physical mixture of the drug with polymer and microspheres were determined using BrukerAXS D8 Advance diffractometer with a radius of 240 mm. The Cu Kα radiation (Cu, Wavelength 1.5406 Å) was Si (Li) filtered. A system of diverging and receiving slits of 1° and 0.1 mm, respectively, was used. The pattern was collected with 40 kV of tube voltage and 30 mA of tube current and scanned over the 20 range of 3°–80°.

In vitro drug diffusion study
The in vitro drug diffusion study of microspheres was performed by Franz diffusion cell (receptor capacity: 17.0 ml; permeation area 3.14 cm²) and dialysis membrane (Mw cut-off 12000–14000) as diffusion barrier. The membrane was equilibrated with phosphate buffer solution (pH 6.6) before dispersing the microspheres into the donor compartment. Microspheres equivalent to 10 mg of pregabalin were applied evenly across the pre-hydrated dialysis membrane. Since the solubility of pregabalin in phosphate buffer (pH 6.6) was 1.287 ± 0.02 mg/ml, sink condition could be maintained. The receptor compartment was filled with 17 ml of phosphate buffer solution (pH 6.6) that was within the pH range in the nasal cavity. The temperature was maintained at 37 ± 1°C using circulating water bath and was stirred with a magnetic stirring bar. A volume of 300 µl of sample was withdrawn at predetermined time points (10 min interval) from the receptor compartment, replaced with the same amount of fresh pre-warmed buffer solution and assayed using UV-visible spectrophotometer. The release studies were carried out in triplicate, and the results were expressed as mean ± SD.[13]

Release kinetics and mechanism of drug release
To study the release kinetics of optimized formulation (P2), data obtained from in vitro drug diffusion studies were plotted in various kinetic models: Zero order (equation 1), first order (equation 2) and Higuchi’s model (equation 3).

\[ C = K_0t \]  
\[ \log C = \log C_0 – Kt/2.303 \]

Where, \( K_0 \) is the zero-order rate constant expressed in units of concentration/time and t the time in minutes. A graph of concentration versus time would yield a straight line with a slope equal to \( K_0 \) and intercept the origin of the axes.

Where \( C_0 \) is the initial concentration of drug, \( K \) the first order rate constant and t the time in minutes.

\[ Qt = Kt^{1/2} \]  
\[ Mt/M_\infty = Kt^n \]

Where \( Mt \) represents the amount of the released drug at time t, \( M_\infty \) the total amount of drug released after an infinite time, \( K \) the diffusional characteristic of the drug-polymer system constant and \( n \) is an exponent that characterizes the mechanism of drug release. The value of \( n \) indicates the drug release mechanism from the delivery system. If the exponent \( n = 0.5 \), then the drug release mechanism is Fickian diffusion, \( n < 0.5 \), the mechanism is quasi-Fickian diffusion, \( n = 0.5–1.0 \), then it is non-Fickian or anomalous diffusion, \( n = 1.0 \), the mechanism is nonFickian Case II diffusion and \( n > 1.0 \), then the mechanism is nonFickian super Case II diffusion.[14]

Ex vivo drug permeation study
The ex vivo permeation study was performed using Franz diffusion cell (receptor capacity: 17.0 ml; permeation area 3.14 cm²) and sheep nasal mucosa as a prototypical membrane. The freshly excised sheep nasal mucosa was collected from the local slaughterhouse, within 1 h of sacrificing the animal and thoroughly cleaned with isotonic saline solution. It was then placed in the diffusion chamber with mucosal surface directed toward donor compartment and serosal surfaces toward receptor compartments. Other experimental and sample collection procedures were performed similarly as in vitro diffusion studies.

Ex vivo biocompatibility study
The mucosal toxicity study was performed to ensure the biocompatibility of pregabalin-loaded CPF with the nasal mucosa. Sheep nasal mucosa was used due to its sensitivity than other tissue. The freshly excised and cleaned sheep nasal mucosa was used for the study. A total of 100 mg of microspheres were applied on to the nasal mucosa. After 1 h of application, the nasal mucosa was fixed in 10% neutral carbonated buffered formalin solution, routinely processed and embedded in paraffin. To assure optimal conditions for
the viability of the tissue, the experiment was carried out in a cell culture incubator (Sanyo Incubator, Model MCO-5AC, Japan). Paraffin sections (7.5 µm) were stained with Hematoxylin-Eosin (HE) and observed under motic microscope. The untreated mucosa directly fixed after isolation was used as a control.\textsuperscript{[15]}

**Pharmacodynamic studies in mice**

For IN administration, animals were held in a supine position under light ketamine (100 mg/kg)/xylazine (10 mg/kg) anesthesia. IN dose (pregabalin 4 mg/kg divided evenly between both nostrils) of microsphere suspension was 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 a suspension of pregabalin loaded CPF (4 mg/kg of pregabalin) \textit{via} intraperitoneal (IP) route. 30 min following drug administration, animals were subcutaneously injected with pentylenetetrazole (PTZ), 80 mg/kg and the onset of clonic-tonic convulsions and percentage protection against mortality in each group was recorded.

**Stability study**

The pregabalin-loaded CPF were placed into borosilicate glass vials and sealed to reduce potential detrimental effects of atmospheric oxygen. Samples were stored at room temperature, 25 ± 2°C with 40 ± 5% RH and 40 ± 2°C over 3 months. After the expiration of these terms, microspheres were analyzed for the determination of particle size, entrapment efficiency, and percent bioadhesion.

**Statistical Analysis**

Statistical analysis was performed using a student \textit{t}-test at the \( P < 0.05 \) level. All the experiments in the study were repeated at least three times, and all data were represented as the mean ± SD. In animal studies, results were analyzed using one-way ANOVA followed by Bonferroni’s multiple comparison test \( P < 0.05 \) was considered statistically significant.

**RESULTS AND DISCUSSION**

In the present study, mucoadhesive microspheres of pregabalin were prepared for IN administration. IN delivery, bypass the BBB and directly target therapeutic agents to the CNS\textsuperscript{[16]} of rodents and humans.\textsuperscript{[17]} Such directed targeting of pregabalin to the brain can avoid gastrointestinal uptake of oral therapy and may permit more potent relief of epilepsy while limiting systemic side effects. For neuroinflammatory, neurodegenerative, and neurovascular disorders,\textsuperscript{[16]} IN delivery is an attractive noninvasive method to target molecules to the CNS.\textsuperscript{[18,19]}

Calibration curve for pregabalin was obtained by plotting concentration versus absorbance of pregabalin. Calibration curve was linear in the range of 2–14 µg/ml and the equation was \( y = 0.005x + 0.001 \) \( (R^2 = 0.997) \). Microspheres were prepared by ionotropic gelation method as it circumvents the use of organic solvents\textsuperscript{[20]} or surfactants commonly used in emulsification and solvent evaporation method.\textsuperscript{[21]} Initially, concentration of acetic acid was selected for the preparation of solution of chitosan. In 0.5% v/v chitosan was only slightly soluble, whereas in 1% v/v acetic acid, it was completely soluble, and hence, it was selected for the preparation of chitosan solution.

**Characterization of Pregabalin Loaded Microspheres**

**Surface morphology**

Figure 1b shows SEM photographs of pregabalin loaded CPF. The surface morphology observed by SEM showed spherical shape and smooth surface of pregabalin-loaded CPF containing 2% w/v of chitosan, noteworthy that they were well prepared by ionotropic gelation method. However with 1% w/v of chitosan particles obtained were not spherical as shown in Figure 1a, whereas further increase in chitosan concentration above 2% w/v leads to formation of aggregated, and larger microspheres might be due to increased viscosity of the polymer solution making stirring insufficient to form smaller and discrete particles.

**Physicochemical properties**

The results of particle size, drug content, entrapment efficiency, percent bioadhesion, and swelling index are shown in Table 2. With 1% w/v chitosan concentration, the particles obtained were irregular with mean size 24.35 ± 1.62 µm, which decreased to 22.32 ± 1.08 µm for microspheres prepared with 2% w/v chitosan. This resultant particle size found to be suitable for nasal administration,\textsuperscript{[14]} further increase in chitosan concentration, increased particle size was observed 37.43 ± 2.43 µm which were more aggregated at 4% w/v of chitosan concentration.\textsuperscript{[8]} As the volume of glutaraldehyde was increased, decrease in the particle size of microspheres was observed. A formulation containing 2% of glutaraldehyde (P2) showed 22.32 ± 1.08 µm particle size which decreased to 20.1 ± 1.25 and 18.4 ± 1.40 µm for the formulations containing 3% (P7) and 5% (P8) of glutaraldehyde, respectively. The stirring speed was also found to affect the size of dispersed droplets. The particle size of the microspheres prepared at 800 rpm was found to be 22.32 ± 1.08 µm which decreased to 20.8 ± 0.96 µm and 18.2 ± 0.87 µm when prepared by increasing the stirring speed at 1000 and 1200 rpm, respectively.

**Drug content and entrapment efficiency**

Drug content ranged from 25.94 to 41.52%, yet all the microspheres had high drug entrapment efficiency between 50.91 and 84.34% depending...
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on the composition of formulations as illustrated in Table 2. The drug entrapment in the CPF containing pregabalin was found to increase as the drug-polymer ratio increased, i.e., as the concentration of chitosan increased the entrapment efficiency was found to decrease. Drug entrapment was observed maximum for the batch P1, P5 and P6, however, the particles were not spherical, so these were batches were not considered for further evaluation whereas batch P2 with 80.64% of entrapment efficiency was selected. Drug entrapment efficiency was also found to decrease with increase in the concentration of crosslinking agent and stirring speed might be due to a decrease in particle sizeable to entrap less amount of drug.

**Ex vivo bioadhesion study**

The *ex vivo* bioadhesion studies of pregabalin-loaded CPF for each formulations was performed using freshly excised sheep nasal mucosa and the percent bioadhesion after 1 h (%) is presented in Table 2. The prepared microspheres showed good bioadhesion strength ranging from 62.18 ± 1.29 to 85.14 ± 1.83%. As observed from results, chitosan concentration showed significant influence on bioadhesive capability of microspheres. It is also widely accepted that the particle with surface charge density like chitosan can serve as good mucoadhesive agents. This is speculated by several earlier findings employing chitosan as polymer for preparation of microsphere for nasal drug delivery system. Batches prepared with the increased amount of glutaraldehyde showed a reduction in bioadhesion potential might be due to more crosslinking of cationic amino groups of chitosan responsible for bioadhesion with negatively charged sialic acid or sulfonic acids of the mucous membrane.

**In vitro swelling study**

The chitosan ratio in microspheres had a direct effect on the swelling ability in phosphate buffer (pH 6.6). As the ratio of chitosan to drug increased, the swelling index increased significantly (P < 0.05). The % equilibrium water uptake of the microspheres was ranging from 85.09 ± 1.43 to 140.7 ± 1.78% 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 a higher concentration of the crosslinking agent. Hence, the crosslinking of microspheres has a great influence on the equilibrium water uptake.

![Figure 1a: Scanning electron microscopy images of pregabalin-loaded chitosan (1% w/v) microspheres](image)

![Figure 1b: Scanning electron microscopy images of pregabalin-loaded chitosan (2% w/v) microspheres](image)
**DSC**

The thermograms of pregabalin, chitosan and pregabalin-loaded CPF are shown in Figure 2. DSC thermogram of pregabalin illustrated sharp endothermic peak 197.54°C, due to its melting point. On the other hand, the DSC thermogram of plain chitosan exhibited a broad endothermic peak at 85°C. By investigating the thermogram of pregabalin loaded CPF (P2), it was found that the endothermic peak was shifted from 85°C to 96°C; this was also associated with the disappearance of the melting peak of pregabalin from the thermogram. This could be attributed to the incorporation and molecular dispersion of pregabalin in chitosan polymer matrices of the prepared microspheres formulations.

**X-ray diffraction study**

The characteristic XRD spectra of pure pregabalin, physical mixture of pregabalin and chitosan and pregabalin-loaded chitosan–microspheres are presented in Figure 3a-c, respectively. Characteristic crystalline peaks of pregabalin were observed in the pure drug sample indicating the presence of crystalline pregabalin. Typical diffractogram showed peaks of pregabalin crystals were present in the physical mixture of pregabalin and chitosan but totally absent in drug-loaded CPF. This showed that pregabalin might be present in the amorphous form after its entrapment in the chitosan matrix.

**In vitro drug diffusion study**

The release of pregabalin from microspheres was immediate with burst effect which might be due to the

![Figure 2: Differential scanning calorimetry thermogram of pure pregabalin, chitosan and pregabalin-loaded chitosan microspheres](image1)

![Figure 3: X-ray diffractogram of (a) pure pregabalin, (b) physical mixture of pregabalin and chitosan and (c) pregabalin-loaded chitosan microspheres](image2)
swelling of polymer and dissolution of the drug in the diffusion medium. The rate and extent of drug release from microspheres were significantly retarded with an increase in chitosan concentration. The release from P2 batch was relatively high compared to other batches [Figure 4]. This is particularly important for prompt absorption of pregabalin to reach the desired drug concentration in plasma after nasal administration. Microspheres of batch P2 showed 94.36 ± 1.46% releases at 100 min having the smallest particle size and maximum entrapment efficiency as compared to other batches. It can be concluded that drug release varies with the concentration of chitosan as it affects particle size as well as entrapment of pregabalin into the microspheres. The release of pregabalin from the microspheres also changes with the crosslinking of polymer. Increase in the amount of crosslinking agent reduces the drug release. Hence, the rate of drug release from the microspheres prepared by ionotropic gelation method can be modified by altering the concentration of chitosan and crosslinking agent glutaraldehyde.

**Drug release kinetics and mechanism**

Results of drug diffusion study of the P2 batch were fitted to kinetic models to investigate the kinetics of drug release. The regression coefficient (R²) for zero order, first order, and Higuchi’s kinetic model was found to be 0.849, 0.948, and 0.989, respectively [Table 3]. Hence, the best-fitted model was found to be Higuchi’s model as it showed the highest value near to linearity. Release data were also fitted to Peppas exponential model, to determine the mechanism of drug release from microspheres formulation. The corresponding plot of Korsmeyer–Peppas’s model indicated a good linearity of regression coefficients (R² = 0.994) and release exponent (n) was found to be 0.5763. The n > 0.5 indicated that the optimized P2 formulation followed the non-Fickian or anomalous diffusion mechanism of drug release.

**Ex vivo permeation study**

The ex vivo permeation study was performed for the optimized formulation P2 due to its favorable particle size, high entrapment efficiency (above 90%) as well as fast release rate. The percent drug permeation of across sheep nasal mucosa from microspheres of batch P2 was found to be 87.29 ± 1.42% after 100 min as represented in Figure 5.

**Ex vivo biocompatibility study**

It is important to maintain the nasal mucosal integrity while preparation of nasal microspheres as repeated exposure may lead to safety issue of the nasal membrane. Figure 6 illustrates the histopathological specimen of untreated (control) nasal mucosa and treated with pregabalin-loaded CPF nasal mucosa stained with HE. No significant changes, sign of damage such as epithelial necrosis or sloughing of epithelial cells was observed on both the specimen. Thus, the result confirmed the biocompatibility of pregabalin loaded CPF with sheep nasal mucosa. Earlier reports also illustrated that chitosan as the least toxic polymer due to its high degree of deacetylation and can be applied to the nasal epithelium.[24]

**Pharmacodynamic Study**

As shown in Table 4, administration of PTZ to vehicle-treated mice produced clonic convulsions in all animals, and the onset of such convulsions was 77.67 ± 2.5 s. IN administration of pregabalin-loaded CPF significantly delayed the onset clonic-tonic...
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Stability Study

The results of stability study are shown in Table 5. It was observed that the particles did not show any changes in their morphology also the microspheres were uniform in size. Microspheres showed a very slight decrease in percent bioadhesion when stored at room temperature, whereas entrapment efficiency was decreased from 92.23 ± 1.02 to 89.17 ± 0.67% when stored at 40 ± 2°C, 75 ± 5% RH. No significant decrease in bioadhesion strength was observed at this storage condition. The changes were negligible enough to conclude that the drug was retained within the microspheres and formulation was found to be stable throughout the stability period.

CONCLUSION

In the present study, pregabalin-loaded chitosan mucoadhesive microspheres were formulated for nasal administration employing ionotropic gelation method. The formulation was characterized by entrapment efficiency, particle size, bioadhesion potential, ex vivo biocompatibility, in vitro drug diffusion profile and ex vivo permeation study. Physicochemical investigations demonstrated that the pregabalin 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 a good permeability of pregabalin-loaded CPF. In animal studies, pre-administration of IN pregabalin-loaded CPF significantly delayed the onset of clonic seizures in PTZ injected mice as compared to their peripheral administration. Importantly, IN pregabalin-loaded CPF offered 83.33% protection against the mortality induced by PTZ compared to 50% following its peripheral administration clearly indicating the greater anticonvulsant effect of pregabalin in PTZ-induced seizures. Thus, IN administration of pregabalin via mucoadhesive microspheres may be appropriate and valuable drug delivery system for the chronic and acute attacks of epileptic seizures.

REFERENCES