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Original Article

# Formulation of Pregabalin-Loaded Trimethyl Chitosan Microspheres for Nasal Drug Delivery: *In vitro, ex vivo* and *in vivo* Characterization

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## ABSTRACT

**Background:** Polar drugs make it difficult to cross nasal cells. Trimethyl Chitosan (TMC) microspheres help absorption by opening junctions, increasing drug retention. Pregabalin absorbs well orally but delays crossing the brain, limiting emergency seizure treatment. The present study aimed to develop pregabalin-loaded TMC microspheres for intranasal administration in epilepsy. **Materials and Methods:** The microspheres were prepared by ionotropic gelation method using TMC and glutaraldehyde by varying the formulation and processing parameters and have been characterized for the physicochemical, drug release and pharmacodynamic properties. **Results:** The microspheres' particle size was 10.71- 22.85 µm with a positive zeta potential of 25.1. At 0.6% TMC, particle size was 14.05±1.24 µm, increasing to 22.85±1.48 µm at 1% TMC concentration, suitable for administration in the nasal cavity without the risk of passing to the lower respiratory tract. TP3 batch (1:1 pregabalin: TMC ratio) had 91.64% entrapment efficiency. Over 90% of drug release was achieved in *in vitro* and *ex vivo* studies using sheep nasal mucosa. The bioadhesion potential measured on sheep nasal mucosa reached 86.29±1.41%. Moreover, the excised sheep nasal mucosa exhibited no morphological toxicity, indicating a high level of biocompatibility. The microspheres significantly delayed the commencement of clonic convulsion (210 s) and presented complete protection (100%) against pentylenetetrazol-induced seizures in mice. **Conclusion:** Pregabalin-loaded TMC microspheres were a promising approach for incorporating hydrophilic drugs and have great potential for nasal administration of pregabalin.

**Keywords:** Pregabalin, Trimethyl chitosan, Microspheres, Intranasal drug delivery, Epilepsy.

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## INTRODUCTION

Although desirable in the treatment of epilepsy, drug delivery to the brain is a tough and challenging task due to the strict nature of the BBB. Diverse methodologies have been evaluated to curtail the influence of the BBB in treating CNS diseases, especially in epilepsy.[1,2] Administration through the nasal route offers a logical way to target CNS delivery.[1] Also, it has gathered significant attention as a promising alternative route for drug administration.[3,4] This non-invasive approach presents various advantages, such as a fast onset of action, bypassing first-pass metabolism and the potential for direct drug transport to the brain via the olfactory and trigeminal nerve pathways.[5] These unique characteristics make intranasal drug delivery an attractive option for treating neurological disorders such as epilepsy that require swift and efficient delivery of drugs to the central nervous system.[6]

However, successfully delivering hydrophilic drugs through the nasal route remains challenging. Pregabalin, a hydrophilic drug, faces difficulties crossing the nasal epithelium due to its large molecular size and tight junctions, which limit its absorption through the transcellular pathway. Consequently, achieving therapeutic drug levels in the brain becomes challenging, leading to suboptimal treatment outcomes for neurological conditions.[7,8]

Microencapsulation using biodegradable polymers has emerged as an innovative and effective strategy.[9] Among these polymers, Trimethyl Chitosan (TMC) is a promising candidate for intranasal drug delivery.[10] TMC possesses excellent mucoadhesive properties, allowing prolonged retaining time of formulations in the nasal mucosa. Furthermore, it can transiently open tight junctions in the cellular membranes; facilitating enhanced drug transport across the nasal epithelium.[11-13] Incorporating polar drugs into TMC microspheres can improve absorption by swiftly inducing the opening of tight junctions in cellular membranes.

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This process enhances the duration of contact between the drug and the absorption site.[7,14]

Pregabalin, a hydrophilic drug widely used in the management of neuropathic pain and epilepsy, serves as an excellent model drug for this study.[15] Although pregabalin shows sufficient oral absorption and a greater degree of intrinsic effectiveness to prevent seizure activity in neuronal networks, it delays the commencement of action and duration of anticonvulsant action in comparison to sodium channel-blocking antiepileptic drugs such as phenytoin.[16] It might be due to a delay in the transport of pregabalin through the blood-brain barrier by a specific large neutral alpha-amino acid transporter.[17] Earlier *in vivo* micro dialysis studies carried out by the researchers showed nearly one-tenth of the concentrations of pregabalin in rat brain extracellular fluid than in plasma.[18] It necessitates the requirement of an alternate route of drug administration for effective anticonvulsant activity to pregabalin.

In addressing the prevailing challenges, the research aimed to develop and characterize pregabalin-loaded TMC microspheres for intranasal drug delivery.[19] The mucoadhesive properties of TMC microspheres enhance drug retention time in nasal mucosa, allowing for improved absorption and taking the drug through the olfactory and trigeminal pathways to the brain.[20-22] This approach holds great potential for enhancing the therapeutic efficacy of pregabalin in treating epilepsy.

## MATERIALS AND METHODS

### Materials

Pregabalin was procured from Glenmark Pharmaceutical Ltd., Mumbai. Chitosan was purchased from Hi-media Laboratories, Mumbai, to synthesize TMC and glutaraldehyde 25% from Rankem, Nagpur (Maharashtra). TMC was prepared and characterized as per our earlier paper[23] and used in the current investigation. All other chemicals, reagents and solvents used were of analytical grade.

### Subjects

To carry out the pharmacodynamic studies, adult male Swiss Albino mice were obtained from the National Institute of Nutrition, Hyderabad (India), weighing 22-25 g. Animals were housed in standard laboratory conditions of temperature (23±1°C) and relative humidity (55±5%) at the Animal Centre for Laboratory Research, SKB College of Pharmacy, Kamptee. Animals have free access to food and water. All the experimental protocols were approved by the Institutional Animal Ethics Committee of the SKB College of Pharmacy, Kamptee and performed according to the guidelines of CPCSEA (853/IAEC/17-18/27).

### Drug-Polymer Interaction Studies

The compatibility between the drug (pregabalin) and the polymer (TMC) is essential in ensuring the chemical stability of the drug during microsphere preparation. Fourier-Transform Infrared spectroscopy (FTIR) and Differential Scanning Calorimetry (DSC) investigated potential interactions between pregabalin and TMC.

### Preparation of Microspheres

The ionotropic gelation method was used for the formulation of pregabalin-loaded TMC microspheres along with glutaraldehyde as a crosslinking agent.[24-27] Pregabalin was added to the aqueous TMC solution of various concentrations (Table 1). It was then crosslinked with polyanionic glutaraldehyde added drop-wise through a 21G disposable syringe (30 drops/min) under constant mechanical stirring. Complexation between oppositely charged TMC and glutaraldehyde, ionic gelation occurs, leading to spherical microspheres. Microspheres were then collected by filtration, washed and dried in the oven (Figure 1). To optimize the formulation of pregabalin-loaded TMC microspheres, TMC concentration, the concentration of the crosslinking agent and stirring speed were varied.[28]

### Characterization of Microspheres

#### Physicochemical and Morphological Properties

The prepared microspheres were characterized comprehensively for various physicochemical properties such as particle size,[29-31] Polydispersity Index (PDI) and zeta potential using Zetasizer (Nano ZS90, Malvern Instruments Ltd., Malvern, UK).[32] Scanning electron microscopy (JEOL Model JSM - 6390LV) was used to find out the surface morphology of the microspheres.[29-31,33]

### Drug content and entrapment efficiency

The microspheres were assessed for drug content and entrapment efficiency according to the reported method.[34] Microspheres equal to 10 mg of the drug were dissolved in 20 mL of methanol, used as a common solvent for the drug and TMC and set aside overnight. It was then centrifuged at 560 rpm for 10 min to remove the insoluble residue. Further, it was filtered and 1 mL of filtrate was analyzed for the pregabalin by UV-visible spectrophotometer (Spectro 2060 plus, UV Spectra TM, Analytical Technologies Ltd., Gujarat, India) at 403 nm. Ninhydrin derivatization of pregabalin was done to determine it spectrophotometrically, prepare a calibration curve and determine pregabalin in microspheres.[23] Drug content was calculated as follows:

$$\text{Drug content } \% = Qp/Q \times 100$$

Where,

Qp=amount of drug encapsulated in microspheres.

Q=Weighed quantity of microspheres.

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

$$\text{Entrapment efficiency (E)} = Qp/Qt \times 100$$

## *In vitro* Drug-Release Study

Drug release from the microspheres was assessed using a Franz diffusion cell with a receptor capacity of 12.0 mL and permeation area of 3.14 $cm^2$ with a dialysis membrane (Mw cut-off 12000-14000) as a diffusion barrier. The membrane was equilibrated with pH 6.6 phosphate buffer solution and placed between the donor and receptor compartments. Microspheres accurately weighing 10 mg of pregabalin were applied uniformly over the pre-hydrated dialysis membrane. The temperature was well-maintained at 37°±1°C with a circulating water bath and was stirred continuously. At a predetermined time of 10 min intervals, 300 µL of the sample was taken from the receptor compartment and analyzed at 403 nm for pregabalin. The release studies were carried out in triplicate and the results were expressed as mean±SD.[21]

## Release Kinetics

The *in vitro* drug release kinetics of the pregabalin-loaded TMC microspheres was studied to understand the drug release profile. The drug release profile was plotted as cumulative drug release (%) versus time (hr). The release data was analyzed using mathematical kinetic models, such as zero-order, first-order, Higuchi and Korsmeyer-Peppas, to determine the release mechanism and kinetics.[35-37]

## *Ex vivo* Drug Permeation Study

*Ex vivo* drug permeation of the pregabalin-loaded TMC microspheres was evaluated using sheep nasal mucosa as a prototypical membrane. The freshly excised sheep nasal mucosa was collected from the local slaughterhouse within 1 hr of sacrificing the animal and cleaned thoroughly with isotonic saline solution. It was then placed in the diffusion chamber with a mucosal surface directed toward the donor compartment and serosal surfaces toward receptor compartments. Experimental and sample collection procedures were similar to those *in vitro* drug release studies.

## *Ex vivo* Bioadhesion Study

The Falling liquid film technique determined the *ex vivo* adhesion of microspheres to the nasal membrane.[38] A piece of 2

**Table 1: Composition of pregabalin microspheres using TMC.**

| Formulation | Pregabalin mg | TMC mg | Glutaraldehyde mL | Stirring speed rpm |
|---|---|---|---|---|
| TP1 | 100 | 60 | 2 | 800 |
| TP2 | 100 | 80 | 2 | 800 |
| TP3 | 100 | 100 | 2 | 800 |
| TP4 | 200 | 100 | 2 | 800 |
| TP5 | 300 | 100 | 2 | 800 |
| TP6 | 100 | 100 | 3 | 800 |
| TP7 | 100 | 100 | 5 | 800 |
| TP8 | 100 | 100 | 2 | 1000 |
| TP9 | 100 | 100 | 2 | 1200 |

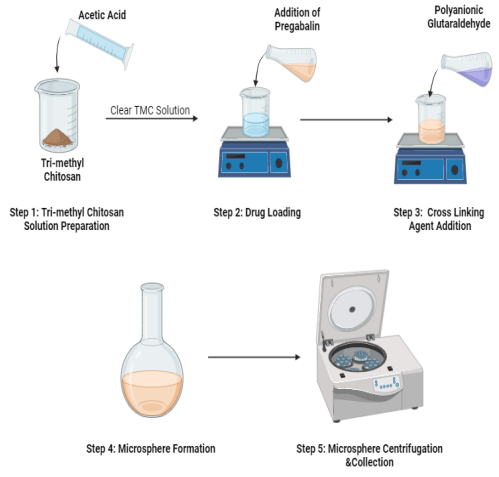


**Figure 1:** Microsphere preparation procedure.

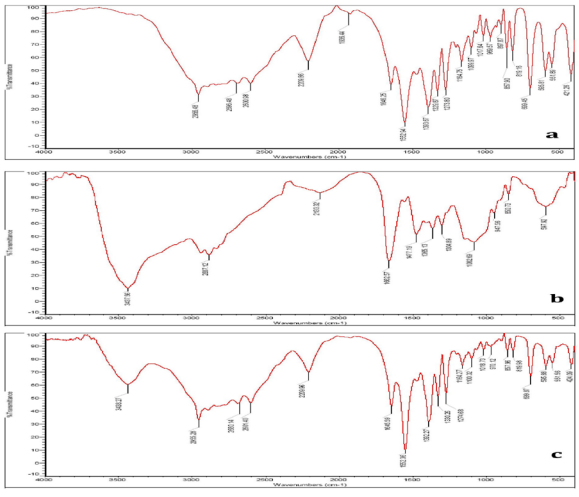


**Figure 2:** FTIR of a) Pregabalin, b) TMC and c) physical mixture of pregabalin and TMC.

$cm^2$ of fresh nasal mucosa was applied with microspheres (100 mg) and then adhered to a polyethylene plate. Approximately 100 mL of simulated nasal electrolyte solution was placed on the microspheres and incubated in desiccators at 90% relative humidity for 15 min to let the polymer interact for adhesion with the membrane. The plate was then kept at a 45° angle to the flat surface, warm phosphate buffer (pH of 6.6) at 37°C was pumped steadily (5 mL/min) over the membrane and the liquid collected in a beaker. After 1 hr, the drug amount in the collected liquid was measured using a spectrophotometer at 403 nm. Microspheres washed off were determined corresponding to the amount of drug present in the perfusate. The amount of adhered microspheres was calculated as the difference between the amount of applied microspheres and the amount of flowed microspheres.[23] The following equation determined the percent mucoadhesion:

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

## *Ex vivo* Biocompatibility Study

A biocompatibility assessment was conducted to ensure the safety of the pregabalin-loaded TMC microspheres on nasal mucosa using excised sheep nasal mucosa. Accurately, 100 mg of microspheres were applied onto the cleaned nasal mucosa; past 1 hr, the nasal mucosa was fixed in 10% neutral carbonated buffered formalin solution, processed routinely and fixed in paraffin. The treated and untreated nasal mucosa was subjected to a cell culture incubator (Sanyo Incubator, Model MCO-5AC, Japan). Further paraffin sections of 7.5 µm were taken and stained with Hematoxylin-Eosin (HE) solution and observed under a Motic microscope (Model: DM2500, Leica Microsystems Inc, ButtaloGrove, IL). The treated mucosa was compared with untreated mucosa as a control.[15] Moreover, it was examined closely for any signs of tissue damage resulting from the *ex vivo* permeation technique.[39-42]

## Pharmacodynamic Studies

For the pharmacodynamic study, the animals were divided into three groups ($n$=6). They underwent an overnight fasting period before the experiments and were brought to the laboratory at least 1 hr prior to the commencement of the study. The experiments were conducted within the light cycle, specifically between 9:00 AM and 1:00 PM. Animals were held in a supine position under light ketamine (100 mg/kg)/xylazine (10 mg/kg) anesthesia for Intranasal (IN) administration. The IN dose (pregabalin 4 mg/kg divided equally between both nostrils) of PRG-TMC microsphere suspension was administered by a Hamilton syringe attached to a polyethylene tube. The tube was introduced nearby 5-6 mm into each nostril for the appropriate delivery of the drug into the nasal cavity. In another group, animals were injected Intraperitoneally (IP) with a suspension of pregabalin-loaded TMC microspheres (4 mg/kg of pregabalin). 30 min post-drug administration, animals were subcutaneously injected with Pentylenetetrazole (PTZ) at a dose of 80 mg/kg. The onset of clonic-tonic convulsions and the percentage of protection against mortality were then recorded for each group.

## Statistical Analysis

Statistical analysis utilized a student t-test at a significance level of $p<0.05$. The experiments were replicated a minimum of three times and the data were expressed as the mean±Standard Deviation (SD). The results were assessed for animal studies through one-way ANOVA, followed by Bonferroni's multiple comparison tests, with statistical significance set at $p<0.05$.

**Table 2: Characterization parameters of pregabalin-loaded TMC microspheres.**

| Formulation code | Percent Yield* (%±SD) | Particle size # (µm±SD) | Drug content* (%±SD) | Encapsulation Efficiency* (%±SD) | Percent Bioadhesion* (%±SD) |
|---|---|---|---|---|---|
| TP1 | 58.25±1.4 | 14.05±1.24 | 39.9±0.88 | 75.04±2.06 | 76.21±1.24 |
| TP2 | 69.73±1.53 | 16.25±1.08 | 41.63±0.96 | 90.24±1.08 | 80.01±1.08 |
| TP3 | 72.90±0.87 | 20.51±1.22 | 42.65±1.03 | 91.64±0.75 | 86.29±1.41 |
| TP4 | 63.10±0.85 | 21.66±1.52 | 38.39±1.22 | 84.10±1.321 | 83.04±1.81 |
| TP5 | 70.22±1.92 | 22.85±1.48 | 40.53±1.54 | 90.34±1.035 | 80.34±1.44 |
| TP6 | 68.66±1.07 | 15.53±1.07 | 40.23±1.51 | 85.04±1.54 | 72.64±1.23 |
| TP7 | 64.23±1.15 | 14.14±0.95 | 37.7±1.07 | 82.02±1.04 | 65.98±1.09 |
| TP8 | 62.96±2.09 | 11.38±1.87 | 41.02±0.85 | 72.61±1.72 | 78.99±1.15 |
| TP9 | 52.10±0.74 | 10.71±1.59 | 38.05±0.68 | 68.68±1.61 | 81.51±0.79 |

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

# RESULTS

## Drug-Polymer Compatibility Studies

The FTIR analysis was carried out to determine the interaction between TMC and Pregabalin (Figure 2). The pregabalin spectrum exhibited a distinctive absorption band at 2956.48 $cm^{-1}$ (C-Stretching), 1552.94 $cm^{-1}$ (N-H stretching) and 1648.25 $cm^{-1}$ (C=O stretching). The FTIR spectra of the physical mixture of pregabalin and TMC show significant peaks of the pure drug and TMC, suggesting the compatibility between pregabalin and TMC. Moreover, the thermogram of the physical mixture of pregabalin and TMC (Figure 3) revealed an endothermic peak at the temperature corresponding to its melting point, supporting the results of FTIR. The XRD spectrum of a mixture of pregabalin and TMC showed all the characteristic diffraction peaks of crystalline PRG, showing the compatibility of pregabalin with TMC (Figure 4).

## Physicochemical and Morphological Characterization of Microspheres

Table 2 shows the percentage yield, particle size, drug content, entrapment efficiency and percent bioadhesion for different batches of microspheres. The percentage yield of microspheres reflects the effectiveness of the microsphere preparation process. The results indicated percentage yields ranging from 52.1% to 72.9%. The particle size of microspheres changes with the concentration of TMC utilized. At 0.6% TMC concentration, the particle size of microspheres was 14.05±1.24 µm, which increased to 16.25±1.08 µm to 20.51±1.22 µm for 0.8% and 1% of TMC concentration, respectively. The resulting particle is appropriate

**Table 3: Zeta potential and PDI of pregabalin-loaded TMC microspheres.**

| Formulation | Zeta potential* Mean±SD | PDI |
|---|---|---|
| TP1 | 22.3±0.18 | 0.223 |
| TP2 | 25.1±0.82 | 0.236 |

**Table 4: Release kinetics of pregabalin-loaded TMC microspheres.**

| Batch | Zero-order | First-order | Higuchi | Hixson. Crow | Korsmeyer-Peppas | |
|---|---|---|---|---|---|---|
| | R | R | R | R | R | n |
| TP2 | 0.9685 | 0.9637 | 0.9928 | 0.9745 | 0.9584 | 0.8165 |
| TP3 | 0.8775 | 0.9488 | 0.9894 | 0.9297 | 0.9873 | 0.5875 |

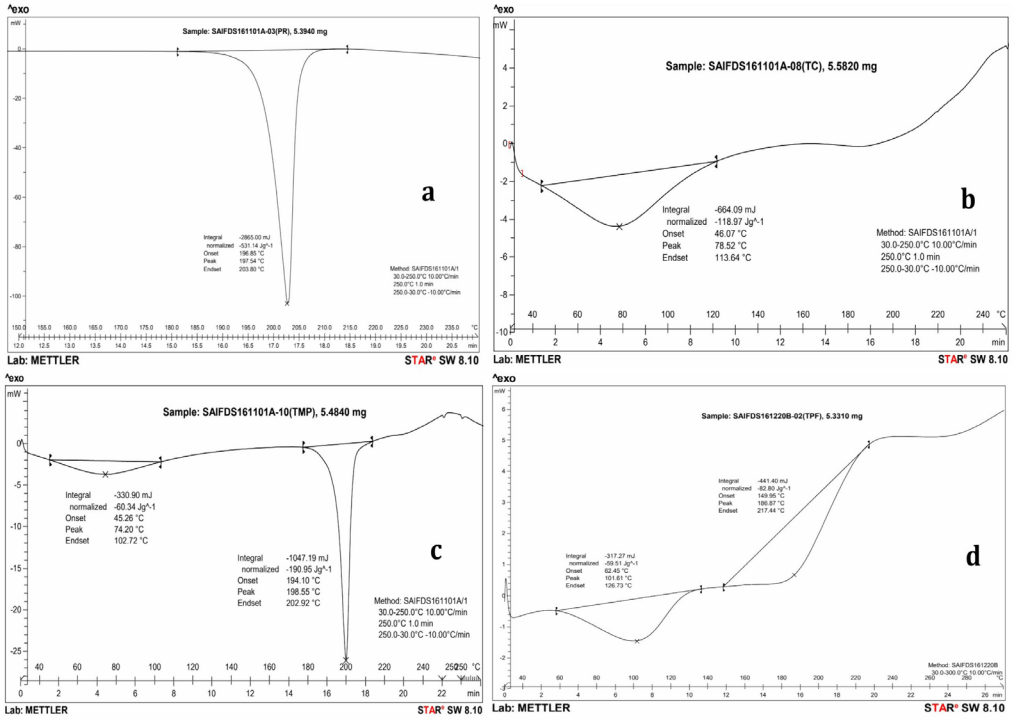


**Figure 3:** DSC thermogram of a) Pregabalin, b) TMC, c) physical mixture of pregabalin and TMC and d) Formulation of pregabalin-TMC.

**Table 5:** Onset of tonic-clonic convulsion and % protection in PTZ-induced mortality in mice.

| | Treatment | Onset of tonic-clonic convulsion (Sec) | % Protection |
|---|---|---|---|
| | **Control** | **77.67±2.5** | **16.66%** |
| TMC Microspheres | TMC/PRG IP | 133.7±2.5* | 50% |
| | TMC/PRG Intra nasal | 210.0±4.09*# | 100% |

*$p$<0.0001 as compared to the Control group; # $p$<0.0001 as compared to the intraperitoneal injected group (ONE WAY ANOVA followed by Bonferroni's multiple comparison test) ($n$=6).

**Table 6:** Results of stability studies of pregabalin-loaded TMC microspheres (TP3) at various storage conditions for 90 days.

| Time in months | Room Temperature | | | 40±2°C, 75±5% RH | | |
|---|---|---|---|---|---|---|
| | Particle size# µm±SD | Entrapment Efficiency* mean±SD | Percent Bioadhesion* mean±SD | Particle size# µm±SD | Entrapment Efficiency* mean±SD | Percent Bioadhesion*mean±SD |
| 0 | 10.7±1.5 | 95.6±1.61 | 81.5±0.7 | 10.7±1.4 | 95.68±1.61 | 81.5±0.79 |
| 30 | 11.2±0.72 | 95.4±0.68 | 81.2±1.12 | 11.3±0.07 | 94.94±0.85 | 80.8±0.27 |
| 60 | 11.5 ±0.09 | 95.0±1.41 | 80.7±1.08 | 11.8±1.05 | 94.73±1.05 | 80.3±1.23 |
| 90 | 11.9 ±0.87 | 94.7±0.08 | 80.1±0.61 | 12.4±0.46 | 94.36±1.07 | 79.7±0.59 |

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

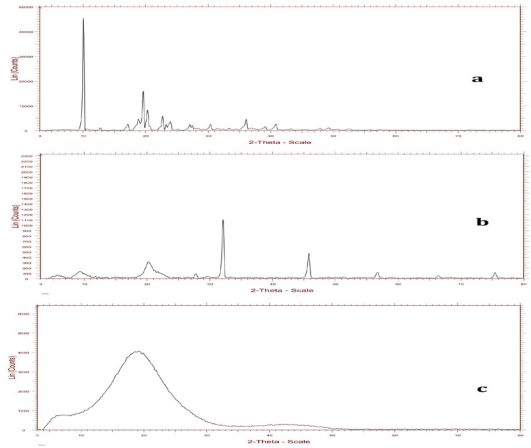


**Figure 4:** XRD Diffractogram of a) Pregabalin, b) TMC and c) Formulation of pregabalin-TMC.

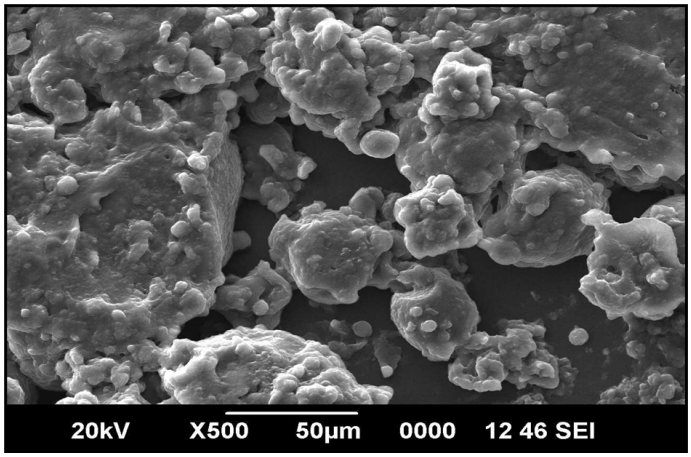


**Figure 5:** SEM image of pregabalin-loaded TMC microspheres.

for deposition in the nasal cavity, minimizing the risk of passage to the lower respiratory tract.[43-44] A notable reduction in particle size was observed with an increase in stirring rate from 800 to 1000 rpm. However, further increments in the stirring rate did not result in a significant decrease in particle size. The average particle sizes of microspheres produced using 2 mL, 3 mL and 5 mL of glutaraldehyde were 20.51±1.22, 15.53±1.07 and 14.14±0.95 µm, respectively. Prepared microspheres showed drug content ranging from 37.7±1.07 to 42.65±1.03%. The encapsulation efficiency was amplified as the pregabalin: TMC ratio decreased. Significantly, TP2 and TP3 batches, formulated with pregabalin: TMC ratio of (1:0.8) and (1:1), exhibited enhanced entrapment efficiencies of 90.24±1.08% and 91.64±0.75%, respectively. As assessed on excised sheep nasal mucosa, the prepared microspheres demonstrated substantial bio-adhesion strength, ranging from 65.98±1.09% to 86.29±1.41%.

## Morphology

Scanning Electron Microscopy (SEM) images of the microspheres provided insights into their morphology. As depicted in Figure 5 the microspheres exhibited well-defined spherical shapes with smooth surfaces, indicating successful preparation of the microspheres by ionotropic gelation technique. The smooth surface is advantageous for mucoadhesion, as it enhances the interaction with the nasal mucosa and promotes prolonged drug residence time. The SEM analysis confirms the desired morphology of the microspheres, which is crucial for their nasal drug delivery application.

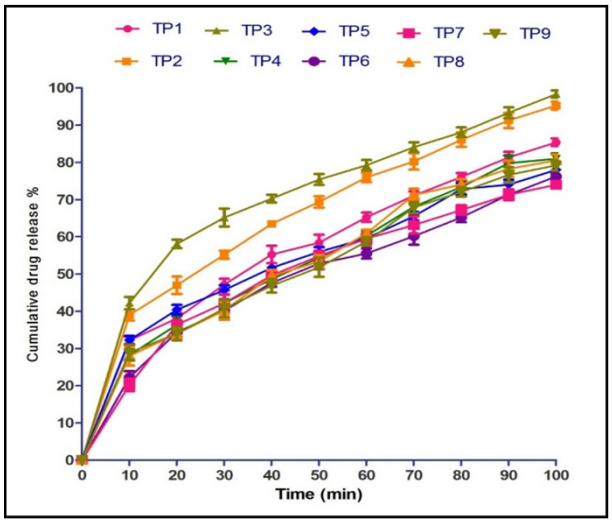


**Figure 6:** *In vitro* drug diffusion profile of pregabalin-loaded TMC microspheres.

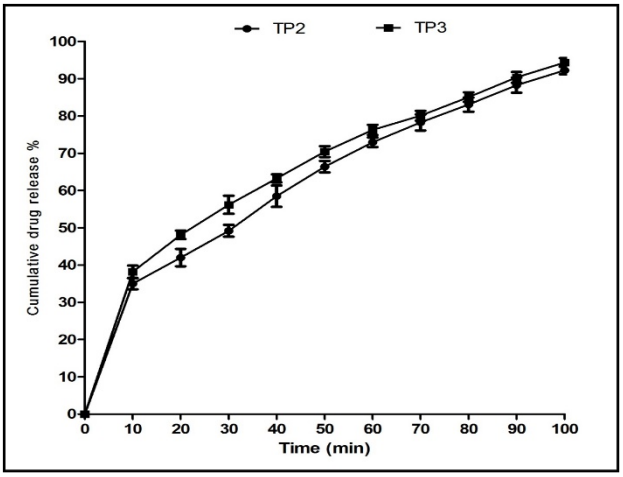


**Figure 7:** *Ex vivo* drug diffusion of pregabalin-loaded TMC microspheres.

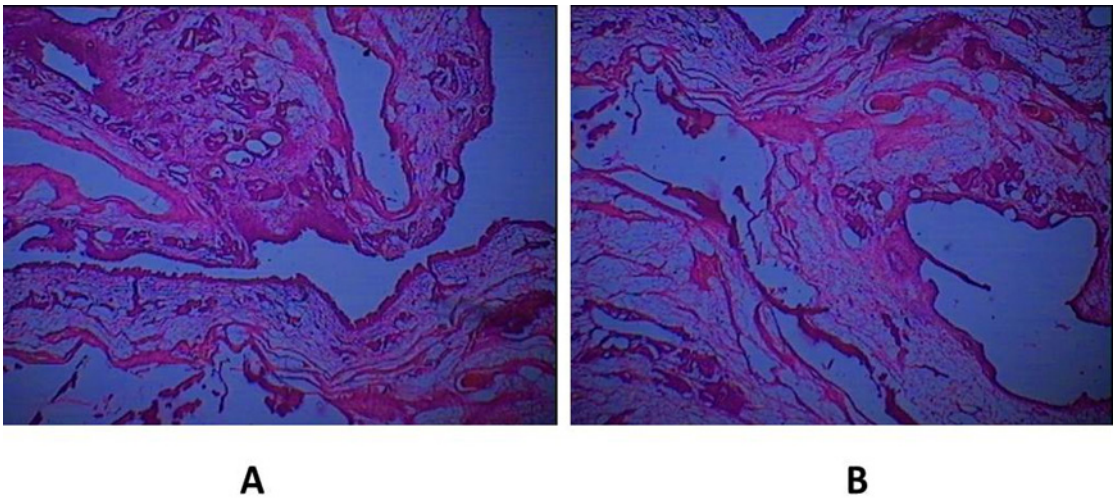


**Figure 8:** Histopathological microphotograph of A) Untreated sheep nasal mucosa (control) B) Pregabalin-loaded TMC microspheres treated sheep nasal.

## Zeta Potential and PDI

Zeta potential and Polydispersity Index (PDI) are important parameters reflecting the microspheres' mucoadhesive properties and particle size distribution. The zeta potential and PDI of the prepared microspheres of pregabalin using TMC were found to be 22.3±0.18/0.223 and 25.1±0.82/0.236 (Table 3). The higher positive zeta potentials observed in the microspheres indicate favorable mucoadhesive properties, facilitating their adherence to the nasal mucosa. The observed interaction is ascribed to the electrostatic interaction between the cationic amino groups of TMC and the anionic moieties present in nasal mucus. The low PDI values also signify a uniform particle size distribution, ensuring reliable drug delivery from the microspheres.

## Characterization of Microspheres by FTIR, DSC and XRD Analysis

DSC and X-ray Diffraction (XRD) analyses further confirmed the successful incorporation of pregabalin into the TMC microspheres. The DSC thermograms (Figure 3) of the drug-loaded microspheres exhibited peaks corresponding to pregabalin, indicating its presence within the microspheres. The disappearance of specific peaks in the XRD pattern of the drug-loaded microspheres indicates the amorphous nature of the drug within the TMC microspheres (Figure 4). The amorphous drug state enhances drug solubility, favoring improved drug release from the microspheres.

### *In vitro* Drug Diffusion and *ex vivo* Drug Permeation Study

The *in vitro* drug diffusion study assessed the release profile of pregabalin from the microspheres. The outcomes of *in vitro* drug diffusion studies revealed that approximately 40-60% of the drug diffused within the first 30 min, indicating an initial burst effect. About 90% of the drug was released at the end of 100 min (Figure 6). However, an initial burst release was observed, which may be attributed to excess drug on the microsphere surface. The optimized batches were further subjected to *ex vivo* permeation studies to assess the ability of the microspheres to facilitate drug absorption through the nasal mucosa. The results indicated enhanced drug permeation across the excised sheep nasal mucosa, confirming the microspheres' capability for effective drug transport across the nasal epithelium (Figure 7).

### Release Kinetics

Results of drug diffusion studies for the TP2 and TP3 batches were analyzed using kinetic models to understand the drug release kinetics. The Regression coefficient (R2) for TP2 (0.9928) and TP3 (0.9894) was found to be greater for Higuchi's kinetic model, as shown in Table 4. Therefore, the formulations follow Higuchi's kinetics, as they exhibited the highest degree of linearity when fitted to the experimental data. The release data were also subjected to fitting with the Peppas exponential model to ascertain the mechanism of drug release from the microsphere's formulation. The values of regression coefficients in the Korsmeyer-Peppas model demonstrated favorable linearity and the release exponent (n) exceeding 0.5 signified that the optimized formulations adhered to a non-Fickian or anomalous diffusion mechanism for drug release.

### *Ex vivo* Biocompatibility Studies

*Ex vivo* biocompatibility studies were carried out to determine the safety of microspheres on nasal mucosa. The preservation of the integrity of nasal mucosa and the absence of significant alterations in tissue structure (Figure 8) indicated the biocompatibility of the microspheres. It suggests the safety and potential use of the microspheres as an intranasal drug delivery system.

### Pharmacodynamic Study

As shown in Table 5, the results of pharmacodynamic investigations indicated that the intranasal administration of pregabalin TMC microspheres delayed the commencement of clonic-tonic seizures up to 210.0±4.09 sec as compared to the control group (77.67±2.5). Additionally, it provided complete protection, preventing 100% mortality induced by PTZ, in contrast to a 50% mortality rate observed with the intraperitoneal administration of pregabalin-TMC microspheres. The results unambiguously demonstrated a more significant anticonvulsant effect of pregabalin-loaded TMC microspheres in PTZ-induced seizures when administered intranasally compared to its peripheral administration.

### Stability Study

The stability study results (Table 6) indicated that the microspheres remained physically stable during the study period. No significant decrease in entrapment efficiency and bioadhesion strength was observed at this storage condition. The observed changes were slight, concluding that the drug remained encapsulated within the microspheres and the formulation demonstrated stability throughout the specified stability period.

## DISCUSSION

Compatibility assessments play a critical role in formulation development. The FTIR spectra analysis revealed no significant interactions between pregabalin and TMC, indicating their compatibility in the formulation. This finding was further supported by the thermal analysis, where the endothermic peak at the melting point of pregabalin remained distinct, suggesting no adverse interactions. These results collectively assure the suitability of using pregabalin and TMC together for microsphere formulation. The effectiveness of the microsphere preparation process is reflected in the yield percentage. The obtained particle size is considered optimal for nasal delivery, minimizing the risk of accidental passage to the lower respiratory tract. Morphological analysis via SEM revealed well-defined spherical microspheres with smooth surfaces. This smooth surface morphology is advantageous for mucoadhesion, promoting prolonged interaction with the nasal mucosa and potentially enhancing drug residence time. This desired morphology observed in the SEM analysis aligns well with the expectations for effective nasal drug delivery. The higher positive zeta potentials measured in the microspheres indicate favorable mucoadhesive properties. This suggests a strong electrostatic interaction between the cationic amino groups of TMC and the anionic components present in nasal mucus, facilitating strong microsphere adhesion to the mucosa. Additionally, the low PDI values signify a uniform particle size distribution, which is crucial for consistent drug release and therapeutic efficacy.

The characterization of pregabalin-loaded TMC microspheres revealed the potential of microspheres for intranasal drug delivery. The disappearance of specific peaks in the XRD pattern confirms the amorphous nature of the drug within the microspheres, which is often linked to improved solubility and faster drug release (Figure 4). This is further supported by the *in vitro* and *ex vivo* drug diffusion study, signifying enhanced permeation across excised sheep nasal mucosa (Figure 7). The *ex vivo* biocompatibility studies provided results regarding the safety of the microspheres for nasal administration. Examination of the nasal mucosa revealed no significant alterations in tissue

structure, signifying that the microspheres do not cause harmful interactions with the nasal tissue (Figure 8). Markedly, intranasal delivery of pregabalin loaded TMC microspheres delayed the onset of clonic-tonic seizures up to 210.0±4.09 sec compared to its peripheral administration, suggesting better anticonvulsant effect with intranasal delivery. Also, the microspheres remained physically stable throughout the study, ensuring consistent drug delivery and efficacy. These findings collectively highlight the potential of pregabalin-loaded TMC microspheres as a promising and safe approach for intranasal drug delivery.

## CONCLUSION

The developed pregabalin-loaded TMC microspheres demonstrate great potential as an intranasal drug delivery system for hydrophilic drugs like pregabalin. The microspheres exhibit smooth and spherical structures with excellent mucoadhesive properties, biocompatibility with nasal mucosa and physical stability over time. These characteristics suggest that pregabalin-loaded TMC microspheres could improve drug bioavailability and enhance therapeutic outcomes in neurological disorders. Additional investigations, encompassing *in vivo* assessments and clinical trials, are imperative to confirm the translational viability of these microspheres for potential clinical applications.

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## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The project was approved by Institutional Animal Ethics Committee of Smt. Kishoritai Bhoyar College of Pharmacy, Kamptee (Protocol Approval Number: 853/IAEC/17-18/27).

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**BBB:** Blood Brain Barrier; **CNS:** Central Nervous System; **FTIR:** Fourier Transform Infrared Spectroscopy; **DSC:** Differential Scanning Calorimetry; **HE:** Hematoxylin-Eosin; **IN:** Intranasal; **IP:** Intraperitoneal; **MS:** Microspheres; **PDI:** Polydispersity Index; **PRG:** Pregabalin; **PTZ:** Pentylenetetrazol; **R2:** Regression Coefficient; **SD:** Standard Deviation; **SEM:** Scanning Electron Microscopy; **TMC:** Trimethyl Chitosan; **TP:** Pregabalin trimethyl chitosan microspheres; **XRD:** X-ray Diffraction.

## SUMMARY

The research was aimed to develop and characterize pregabalin-loaded TMC microspheres for nasal administration. The microspheres were prepared using the ionotropic gelation method and characterized for size, zeta potential, entrapment efficiency, drug release, mucoadhesion and biocompatibility study. The microspheres were found to be spherical with smooth surfaces, having a size ranging from 10.71 to 22.85 µm and a positive zeta potential. The entrapment efficiency was high, exceeding 90%. *In vitro* and *ex vivo* studies using sheep nasal mucosa showed that over 90% of the drug was released from the microspheres. The microspheres also exhibited good bioadhesion potential to sheep nasal mucosa and were found to be biocompatible. Furthermore, the microspheres significantly delayed the onset of seizures and provided complete protection against pentylenetetrazol-induced seizures in mice. These findings suggest that pregabalin-loaded TMC microspheres have great potential for nasal delivery of pregabalin and could be a promising approach for the treatment of epilepsy.

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