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INTERPOLYMER COMPLEX-BASED MUCOADHESIVE MICROSPHERES OF PREGABALIN: FORMULATION OPTIMISATION, RELEASE KINETICS, AND GASTRORETENTIVE EVALUATION

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ABSTRACT
The present study aimed to develop and optimize pregabalin-loaded mucoadhesive microspheres using an interpolymer complexation technique for gastroretentive drug delivery. Microspheres were prepared by combining ionotropic gelation with electrostatic interaction between chitosan and sodium alginate. A Box–Behnken Design was employed to systematically investigate the influence of chitosan concentration, alginate concentration, and calcium chloride concentration on critical quality attributes, including particle size, entrapment efficiency, mucoadhesion, and drug release. The optimized formulation exhibited a particle size of 538 ± 22 µm, entrapment efficiency of 85.8 ± 1.7%, and mucoadhesion of 87.6 ± 1.8%, indicating efficient drug encapsulation and strong adhesion potential. In vitro drug release studies demonstrated a sustained release profile, with approximately 85% drug release over 12 hours. Release kinetics analysis revealed that drug release followed the Higuchi model with non-Fickian diffusion behavior. Fourier transform infrared spectroscopy and differential scanning calorimetry confirmed the absence of chemical incompatibility and indicated partial amorphization of the drug within the polymer matrix. Stability studies showed no significant changes in formulation parameters over three months under accelerated conditions. Overall, the developed microspheres represent a promising gastroretentive system for improving the therapeutic performance and patient compliance of pregabalin.

KEYWORDS: Pregabalin; Mucoadhesive microspheres; Interpolymer complexation; Gastroretentive drug delivery; Controlled release; Box–Behnken Design

1. INTRODUCTION
Pregabalin is a widely prescribed antiepileptic and neuropathic pain modulator that exerts its pharmacological action by binding to the voltage-gated calcium channels, thereby reducing excitatory neurotransmitter release. It is clinically indicated in the management of neuropathic pain, epilepsy, fibromyalgia, and generalized anxiety disorders. Despite its favourable pharmacokinetic profile, pregabalin exhibits a relatively short elimination half-life of approximately 5–7 hours, necessitating multiple daily dosing to maintain therapeutic plasma concentrations. This frequent dosing regimen often compromises patient compliance, particularly in chronic conditions requiring long-term therapy. Therefore, the development of a sustained and site-specific drug delivery system for pregabalin remains a significant pharmaceutical objective (Nagao et al., 2022; Sakama et al., 2025; Zhang et al., 2025).

Gastroretentive drug delivery systems (GRDDS) have emerged as an effective strategy to enhance the bioavailability and therapeutic efficacy of drugs by prolonging their residence time in the stomach. These systems are particularly advantageous for drugs that are absorbed primarily in the upper gastrointestinal tract or require controlled release to maintain consistent plasma levels. Among the various GRDDS approaches, mucoadhesive systems have gained considerable attention due to their ability to adhere to the gastric mucosa, thereby resisting gastric emptying and ensuring localized drug delivery over an extended period (Omachi, 2022; Omidian, 2025; Pahwa et al., 2012). Mucoadhesive microspheres represent a promising class of gastroretentive systems that combine the advantages of multiparticulate dosage forms with enhanced mucosal adhesion. These systems provide uniform drug distribution, reduced risk of dose dumping, and improved patient safety compared to single-unit formulations. The selection of appropriate polymers plays a crucial role in determining the performance of mucoadhesive microspheres. Chitosan, a cationic polysaccharide, is widely recognized for its excellent

mucoadhesive properties due to its ability to form electrostatic interactions with negatively charged mucin. Sodium alginate, an anionic polymer, contributes to gel formation and structural integrity through ionic crosslinking with divalent cations such as calcium. The combination of these polymers can lead to the formation of interpolymer complexes with enhanced mechanical strength and functional performance (Panraksa et al., 2025; Srivastava et al., 2021; Turac et al., 2024; Vinchurkar et al., 2022).

Interpolymer complexation (IPC) is a formulation strategy that involves the interaction between oppositely charged polymers to form a stable network through electrostatic and hydrogen bonding interactions. This approach offers several advantages, including improved drug entrapment, enhanced mucoadhesion, and controlled drug release. When combined with ionotropic gelation, IPC can produce microspheres with a dense and cohesive matrix capable of modulating drug diffusion and prolonging release. Despite the potential of this approach, limited studies have explored its application in the development of pregabalin-loaded gastroretentive systems (Sørensen et al., 2021; Southward et al., 2025; Srivastava et al., 2021).

In this context, the present study aimed to formulate and characterize pregabalin-loaded mucoadhesive microspheres using an interpolymer complexation technique. A systematic optimization approach based on Box–Behnken Design was employed to evaluate the influence of formulation variables on key performance parameters. The developed microspheres were subjected to comprehensive physicochemical characterization, in vitro drug release studies, and kinetic modelling to elucidate the release mechanism. The study was designed to establish a robust and effective gastroretentive delivery system for pregabalin, with the potential to improve therapeutic outcomes and patient compliance.

2. MATERIALS AND METHODS

2.1 Materials

Pregabalin was used as the model drug for the development of mucoadhesive gastroretentive microspheres. The active pharmaceutical ingredient (API) was procured from a certified pharmaceutical supplier with a declared purity of not less than 99% and was used without further purification. Chitosan (medium molecular weight, degree of deacetylation approximately 85%) was selected as the primary cationic polymer due to its well-documented mucoadhesive properties and ability to form polyelectrolyte complexes. Sodium alginate, a naturally occurring anionic polysaccharide, was utilized as the counter polymer to facilitate interpolymer complexation and ionotropic gelation. Hydroxypropyl methylcellulose (HPMC K100M) was incorporated as a release-retarding polymer to modulate the drug release profile.

Calcium chloride ($CaCl_2$) was employed as the crosslinking agent to induce gelation of sodium alginate and stabilize the microsphere structure. Glacial acetic acid was used to prepare the chitosan solution (1% v/v aqueous solution), ensuring complete solubilization of the polymer. Phosphate buffer (pH 6.8) was prepared according to pharmacopeial specifications and used as the dissolution medium. All other chemicals and reagents used in the study were of analytical grade and were utilized as received without further purification. Distilled water was used throughout the study.

2.2 Experimental Design and Optimization Strategy

A systematic optimization approach was adopted using Box–Behnken Design (BBD) to evaluate the effect of formulation variables on critical quality attributes of the microspheres. This design was selected due to its efficiency in exploring quadratic response surfaces with a reduced number of experimental runs, while avoiding extreme combinations that may lead to formulation instability. Three independent formulation variables were selected based on preliminary screening studies and literature justification (Pande et al., 2022a; Tambe et al., 2023):

- A: Chitosan concentration (% w/v)
- B: Sodium alginate concentration (% w/v)
- C: Calcium chloride concentration (% w/v)

Each variable was studied at three levels: low (−1), medium (0), and high (+1). The selected levels were fixed as follows:

- Chitosan: 0.5%, 1.0%, 1.5%
- Sodium alginate: 1.0%, 1.5%, 2.0%
- Calcium chloride: 2%, 4%, 6%

A total of 17 experimental runs were generated by the design, including five center points to evaluate experimental error and reproducibility. The responses selected for optimization were:

- Particle size (μm)
- Entrapment efficiency (%)
- Mucoadhesion (%)
- Cumulative drug release at 12 hours (%)

The experimental data obtained from all runs were subjected to multiple regression analysis to generate polynomial equations describing the relationship between independent variables and responses. Analysis of variance (ANOVA) was performed to evaluate the statistical significance of the model and individual factors. Response surface plots and contour plots were generated to visualize the effect of variables and identify the optimized formulation using a desirability function approach (Pande et al., 2022a; Tambe et al., 2023).

2.3 Preformulation Studies

2.3.1 Organoleptic and Physical Characterization of Drug

Pregabalin was initially evaluated for its organoleptic properties, including colour, odour, and physical appearance. The drug was observed to be a white to off-white crystalline powder with no characteristic odour. The physical state and flow properties were noted to ensure suitability for formulation development (Santonocito et al., 2022; Steele & Austin, 2016; Szymańska et al., 2022).

2.3.2 Determination of λmax of Pregabalin

The maximum absorption wavelength (λmax) of pregabalin was determined using a UV-visible spectrophotometer. A stock solution of pregabalin was prepared by dissolving 10 mg of the drug in 100 mL of phosphate buffer (pH 6.8) to obtain a concentration of 100 µg/mL. This solution was further diluted to obtain a working concentration of 10 µg/mL. The solution was scanned over a wavelength range of 200–400 nm against phosphate buffer as blank. The λmax of pregabalin was identified at approximately 210 nm, and this wavelength was selected for all subsequent quantitative analyses (Santonocito et al., 2022; Steele & Austin, 2016; Szymańska et al., 2022).

2.3.3 Preparation of Calibration Curve

A calibration curve of pregabalin was constructed in phosphate buffer (pH 6.8) to enable quantitative estimation during drug content and release studies. Accurately weighed 10 mg of pregabalin was dissolved in 100 mL of phosphate buffer to obtain a stock solution of 100 µg/mL. Aliquots of the stock solution were withdrawn and diluted to obtain concentrations in the range of 2–20 µg/mL. The absorbance of each solution was measured at 210 nm using a UV-visible spectrophotometer. A calibration curve was plotted between concentration and absorbance, and the regression equation along with correlation coefficient (R^2) was determined (Santonocito et al., 2022; Steele & Austin, 2016; Szymańska et al., 2022).

2.3.4 Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectroscopy was performed to investigate possible interactions between pregabalin and selected polymers. The spectra of pure drug, individual polymers (chitosan, sodium alginate, HPMC K100M), and physical mixtures were recorded. Samples were prepared using the potassium bromide (KBr) pellet method. Approximately 1–2 mg of sample was mixed with KBr and compressed into a transparent pellet using a hydraulic press. The spectra were recorded in the range of 4000–400 cm^{-1} using an FTIR spectrophotometer. The characteristic peaks of pregabalin, including functional groups such as amine and carboxyl groups, were identified and compared with those of the physical mixture to assess any shifts, disappearance, or formation of new peaks indicative of chemical interaction (Santonocito et al., 2022; Steele & Austin, 2016; Szymańska et al., 2022).

2.3.5 Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry was performed to evaluate the thermal behaviour and compatibility of pregabalin with selected polymers. Approximately 5 mg of sample (pure drug and optimized formulation) was weighed and sealed in aluminium pans. The samples were heated from 30°C to 300°C at a heating rate of 10°C/min under a nitrogen atmosphere. The thermograms obtained were analyzed for melting point, peak shifts, and changes in enthalpy. The disappearance or broadening of the drug's characteristic melting peak was interpreted as an indication of drug dispersion or amorphization within the polymer matrix (Santonocito et al., 2022; Steele & Austin, 2016; Szymańska et al., 2022).

2.3.6 Solubility Study

The solubility of pregabalin was determined in distilled water and phosphate buffer (pH 6.8) using the shake flask method. An excess amount of drug was added to 10 mL of solvent and shaken at 25°C for 24 hours in a thermostatically controlled shaker. The samples were then filtered through Whatman filter paper, and the filtrate was analyzed spectrophotometrically at 210 nm after appropriate dilution. The solubility values were calculated to ensure that sink conditions could be maintained during dissolution studies (Santonocito et al., 2022; Steele & Austin, 2016; Szymańska et al., 2022).

2.3.7 Drug-Polymer Compatibility Assessment

In addition to FTIR and DSC analysis, the compatibility between pregabalin and polymers was assessed based on physical observation of mixtures stored under accelerated conditions (40°C ± 2°C / 75% RH ± 5%) for a period of 7 days. The samples were evaluated for any changes in colour, odour, or physical consistency, which could indicate potential incompatibility.

2.4 Preparation of Pregabalin-Loaded Mucoadhesive Microspheres

Pregabalin-loaded mucoadhesive microspheres were prepared using a combined ionotropic gelation and interpolymer complexation (IPC) technique, which exploits electrostatic interactions between oppositely charged polymers to form a stable, mucoadhesive matrix (Pande et al., 2022b; Sapra et al., 2024; Sharma et al., 2021; Wang et al., 2025).

2.4.1 Preparation of Polymer–Drug Dispersion

Accurately weighed chitosan was dissolved in 1% v/v aqueous acetic acid solution under continuous magnetic stirring to obtain a clear, homogeneous solution. The concentration of chitosan was varied according to the experimental design (0.5%, 1.0%, or 1.5% w/v). Stirring was maintained at 800 rpm for 2 hours to ensure complete dissolution. Pregabalin was then added to the chitosan solution in a fixed drug-to-polymer ratio (1:2 w/w with respect to total polymer weight) and stirred for 30 minutes to ensure uniform dispersion. Subsequently, sodium

alginate (1.0–2.0% w/v) and HPMC K100M (fixed at 0.5% w/v across all formulations) were gradually incorporated into the drug–polymer mixture under continuous stirring. The dispersion was homogenized at 800 rpm for an additional 30 minutes to achieve a uniform viscous system (Pande et al., 2022b; Sapra et al., 2024; Sharma et al., 2021; Wang et al., 2025).

2.4.2 Formation of Microspheres via Ionotropic Gelation and IPC

The prepared polymer–drug dispersion was transferred into a 10 mL syringe fitted with a 22-gauge needle. The dispersion was then introduced dropwise into a gently stirred calcium chloride solution (2–6% w/v) maintained under constant stirring at 500 rpm. Upon contact with the calcium chloride solution, instantaneous gelation of sodium alginate occurred due to ionic crosslinking with Ca^{2+} ions, resulting in the formation of discrete microspheres. Simultaneously, electrostatic interactions between the positively charged chitosan and negatively charged alginate led to the formation of an interpolymer complex, further stabilizing the microsphere structure and enhancing mucoadhesion (Pande et al., 2022b; Sapra et al., 2024; Sharma et al., 2021; Wang et al., 2025).

2.4.3 Curing, Collection, and Drying of Microspheres

The formed microspheres were allowed to cure in the calcium chloride solution for a fixed duration of 30 minutes to ensure complete crosslinking and structural stabilization. Following curing, the microspheres were collected by filtration and washed three times with distilled water to remove excess calcium ions and unbound drug. The washed microspheres were then dried in a hot air oven at 40°C for 12 hours until constant weight was achieved. The dried microspheres were stored in a desiccator until further evaluation (Pande et al., 2022b; Sapra et al., 2024; Sharma et al., 2021; Wang et al., 2025).

2.5 Evaluation of Microspheres

2.5.1 Percentage Yield

The percentage yield of microspheres was calculated to assess the efficiency of the preparation process. The dried microspheres were weighed, and the yield was calculated using the following equation (Pande et al., 2022b; Sapra et al., 2024; Sharma et al., 2021; Wang et al., 2025):

$$\text{Percentage Yield (\%)} = \frac{\text{Practical weight of microspheres}}{\text{Total theoretical weight of drug and polymers}} \times 100$$

2.5.2 Particle Size Analysis

The particle size of the microspheres was determined using optical microscopy. A small quantity of microspheres was dispersed in distilled water and placed on a glass slide. The diameters of at least 100 microspheres were measured using a calibrated ocular micrometer. The mean particle size was calculated and expressed in micrometers (μm). The distribution pattern was also noted to evaluate uniformity (Pande et al., 2022b; Sapra et al., 2024; Sharma et al., 2021; Wang et al., 2025).

2.5.3 Surface Morphology (SEM Analysis)

The surface morphology and structural characteristics of the microspheres were examined using scanning electron microscopy (SEM). Dried microspheres were mounted on aluminum stubs using double-sided adhesive tape and coated with a thin layer of gold under vacuum. The samples were observed at different magnifications to assess shape, surface smoothness, and presence of pores or cracks, which are critical factors influencing drug release behaviour (Pande et al., 2022b; Sapra et al., 2024; Sharma et al., 2021; Wang et al., 2025).

2.5.4 Entrapment Efficiency (%)

Entrapment efficiency was determined to quantify the amount of drug successfully incorporated into the microspheres. An accurately weighed quantity of microspheres equivalent to 50 mg of drug was crushed and dissolved in 100 mL of phosphate buffer (pH 6.8). The solution was stirred for 2 hours to ensure complete extraction of the drug. The solution was filtered, appropriately diluted, and analyzed spectrophotometrically at 210 nm. Entrapment efficiency was calculated using the following formula (Pande et al., 2022b; Sapra et al., 2024; Sharma et al., 2021; Wang et al., 2025):

$$\text{Entrapment Efficiency (\%)} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$$

2.5.5 Drug Content Uniformity

Drug content was determined using the same procedure as entrapment efficiency but expressed as the percentage of drug present in the microspheres relative to the total weight of microspheres. This parameter ensured uniform distribution of drug within the formulation.

2.5.6 Swelling Index

The swelling behaviour of microspheres was evaluated to understand their hydration characteristics and influence on mucoadhesion. Accurately weighed microspheres were immersed in phosphate buffer (pH 6.8) at 37°C. At predetermined time intervals, the microspheres were removed, blotted to remove excess surface water, and weighed. The swelling index was calculated using the following equation (Pande et al., 2022b; Sapra et al., 2024; Sharma et al., 2021; Wang et al., 2025):

$$\text{Swelling Index (\%)} = \frac{W_t - W_0}{W_0} \times 100$$

where $W_0$is the initial weight and $W_t$is the weight after swelling.

2.5.7 *In Vitro* Mucoadhesion Study (Wash-Off Method)

The mucoadhesive property of the microspheres was evaluated using a wash-off test. Freshly excised rat stomach mucosa was obtained and mounted onto a glass slide using thread. A known quantity of microspheres was spread uniformly over the mucosal surface. The slide was then attached to the arm of a USP disintegration apparatus and subjected to up-and-down movements in phosphate buffer (pH 6.8) maintained at 37°C. At specified time intervals, the number of microspheres remaining adhered to the mucosa was counted. The percentage mucoadhesion was calculated as (Pande et al., 2022b; Sapra et al., 2024; Sharma et al., 2021; Wang et al., 2025):

$$\text{Mucoadhesion (\%)} = \frac{\text{Number of adhered microspheres}}{\text{Total microspheres applied}} \times 100$$

2.5.8 In Vitro Drug Release Study

The drug release profile of the microspheres was evaluated using a USP Type II (paddle) dissolution apparatus. Microspheres equivalent to 100 mg of pregabalin were placed in 900 mL of phosphate buffer (pH 6.8) maintained at 37 ± 0.5°C. The paddle speed was set at 50 rpm. At predetermined intervals (0, 1, 2, 4, 6, 8, 10, and 12 hours), 5 mL samples were withdrawn and replaced with fresh medium to maintain sink conditions. The samples were filtered, diluted, and analyzed spectrophotometrically at 210 nm. The cumulative percentage drug release was calculated and plotted against time (Pande et al., 2022b; Sapra et al., 2024; Sharma et al., 2021; Wang et al., 2025).

2.6 Drug Release Kinetic Modelling

To elucidate the mechanism of drug release from the formulated mucoadhesive microspheres, the in vitro dissolution data were fitted to various kinetic models. The cumulative drug release data obtained over 12 hours were analyzed using zero-order, first-order, Higuchi, and Korsmeyer–Peppas models. Each model was selected to represent a distinct release mechanism and to identify the dominant process governing drug release. The model fitting was performed by transforming the dissolution data into appropriate linear forms, followed by regression analysis. The model showing the highest correlation coefficient (R^2) was considered the best-fit model (Pande et al., 2022b; Sapra et al., 2024; Sharma et al., 2021; Wang et al., 2025).

2.6.1 Zero-Order Kinetics

Zero-order kinetics describes a system in which drug release occurs at a constant rate, independent of drug concentration. The model was evaluated by plotting cumulative percentage drug release versus time.

$$Q_t = Q_0 + k_0 t$$

where $Q_t$represents the amount of drug released at time t, $Q_0$is the initial amount of drug in solution (typically zero), and $k_0$is the zero-order release constant (Pande et al., 2022b; Sapra et al., 2024; Sharma et al., 2021; Wang et al., 2025).

2.6.2 First-Order Kinetics

First-order kinetics assumes that the drug release rate is concentration-dependent. The logarithm of the remaining drug was plotted against time.

$$\log Q_t = \log Q_0 - \frac{k_1 t}{2.303}$$

where $k_1$is the first-order release constant.

2.6.3 Higuchi Model

The Higuchi model describes drug release from a matrix system as a diffusion-controlled process based on Fick's law.

$$Q_t = k_H t^{1/2}$$

where $Q_t$is the amount of drug released at time t, and $k_H$is the Higuchi dissolution constant.

2.6.4 Korsmeyer–Peppas Model

The Korsmeyer–Peppas model was applied to analyze the release mechanism when more than one type of release phenomenon was involved.

$$\frac{M_t}{M_\infty} = kt^n$$

where $M_t/M_\infty$is the fraction of drug released at time t, $k$is the release rate constant, and $n$is the release exponent indicating the mechanism of drug release. The value of $n$was interpreted as follows:

- $n \leq 0.5$: Fickian diffusion
- $0.5 < n < 1.0$: Non-Fickian (anomalous) transport
- $n = 1$: Case II transport

2.7 Statistical Analysis and Model Validation

2.7.1 Regression Analysis

The experimental data obtained from the Box–Behnken Design were analyzed using multiple regression analysis to establish quantitative relationships between formulation variables and responses. A second-order polynomial equation was generated for each response as follows:

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2$$

where Yrepresents the response, $\beta_0$is the intercept, and $\beta_1$to $\beta_{33}$are regression coefficients representing linear, interaction, and quadratic effects.

2.7.2 Analysis of Variance (ANOVA)

ANOVA was performed to evaluate the statistical significance of the developed models and individual terms. The significance was determined based on the F-value and p-value, with a p-value less than 0.05 considered statistically significant.

The following statistical parameters were evaluated:

- Coefficient of determination (R^2)
- Adjusted R^2
- Predicted R^2
- Adequate precision (signal-to-noise ratio)
- Lack-of-fit test

A non-significant lack-of-fit ($p > 0.05$) indicated that the model was suitable for prediction within the studied range.

2.7.3 Response Surface and Contour Plot Analysis

Three-dimensional response surface plots and two-dimensional contour plots were generated to visualize the effect of independent variables on each response. These graphical representations aided in understanding the interaction between formulation variables and identifying optimal conditions.

2.7.4 Optimization Using Desirability Function

A numerical optimization technique based on the desirability function approach was employed to determine the optimal formulation. Each response was assigned a specific goal:

- Particle size: Minimize
- Entrapment efficiency: Maximize
- Mucoadhesion: Maximize
- Drug release: Target (controlled release profile)

The overall desirability value (D) was calculated, and the formulation with the highest desirability was selected as the optimized batch.

2.8 Validation of Optimized Formulation

The optimized formulation predicted by the statistical model was prepared experimentally to validate the accuracy of the model. The observed values of responses were compared with predicted values, and the percentage prediction error was calculated. A low prediction error confirmed the validity and robustness of the developed model.

2.9 Stability Study

The optimized microsphere formulation was subjected to short-term stability studies as per ICH guidelines. The samples were stored in airtight containers at accelerated conditions (40°C ± 2°C and 75% ± 5% relative humidity) for a period of 3 months. At predetermined intervals (0, 1, and 3 months), the samples were evaluated for:

- Particle size
- Entrapment efficiency
- Mucoadhesion
- Drug release profile

Any significant changes in these parameters were noted to assess formulation stability.

3. RESULTS AND DISCUSSION

3.1 Box–Behnken Design Outcomes and Experimental Data

The formulation of pregabalin-loaded mucoadhesive microspheres was systematically optimized using a three-factor, three-level Box–Behnken Design (BBD). A total of 17 experimental runs were generated, and the observed responses, including particle size, entrapment efficiency, mucoadhesion, and cumulative drug release at 12 hours, are presented below.

Table 1: Experimental Design Matrix and Observed Responses

Run	A: Chitosan (%)	B: Alginate (%)	C: $CaCl_2$ (%)	Particle Size (µm)	EE (%)	Mucoadhesion (%)	Drug Release (%)
1	0.5	1.0	4	412 ± 18	71.5 ± 1.8	68.2 ± 2.1	96.4 ± 2.3
2	1.5	1.0	4	528 ± 22	83.6 ± 2.0	82.4 ± 1.9	88.7 ± 2.1
3	0.5	2.0	4	465 ± 20	75.3 ± 1.6	74.6 ± 2.0	92.1 ± 2.4
4	1.5	2.0	4	602 ± 25	89.4 ± 1.7	91.2 ± 1.6	81.3 ± 2.2

5	**0.5**	**1.5**	**2**	**395 ± 16**	**69.8 ± 2.1**	**65.7 ± 2.4**	**97.8 ± 2.5**
6	**1.5**	**1.5**	**2**	**575 ± 24**	**86.9 ± 1.9**	**88.3 ± 1.8**	**85.6 ± 2.3**
7	**0.5**	**1.5**	**6**	**438 ± 19**	**73.5 ± 2.0**	**70.1 ± 2.2**	**94.5 ± 2.6**
8	**1.5**	**1.5**	**6**	**610 ± 26**	**90.2 ± 1.8**	**92.5 ± 1.5**	**79.6 ± 2.0**
9	**1.0**	**1.0**	**2**	**460 ± 18**	**78.2 ± 1.7**	**74.8 ± 2.0**	**92.7 ± 2.3**
10	**1.0**	**2.0**	**2**	**520 ± 21**	**82.6 ± 1.9**	**80.3 ± 1.9**	**88.4 ± 2.1**
11	**1.0**	**1.0**	**6**	**490 ± 20**	**80.4 ± 2.1**	**78.5 ± 2.2**	**90.2 ± 2.4**
12	**1.0**	**2.0**	**6**	**565 ± 23**	**86.1 ± 1.8**	**85.7 ± 1.7**	**83.9 ± 2.2**
13	**1.0**	**1.5**	**4**	**540 ± 22**	**85.2 ± 1.6**	**86.8 ± 1.8**	**84.7 ± 2.0**
14	**1.0**	**1.5**	**4**	**538 ± 21**	**84.9 ± 1.7**	**87.1 ± 1.9**	**85.1 ± 2.1**
15	**1.0**	**1.5**	**4**	**542 ± 23**	**85.4 ± 1.8**	**86.5 ± 1.7**	**84.5 ± 2.3**
16	**1.0**	**1.5**	**4**	**539 ± 22**	**85.0 ± 1.9**	**87.0 ± 1.8**	**84.9 ± 2.2**
17	**1.0**	**1.5**	**4**	**541 ± 21**	**85.3 ± 1.7**	**86.7 ± 1.9**	**84.6 ± 2.1**

The experimental data clearly demonstrated that all four responses were significantly influenced by the selected formulation variables. Particle size ranged from approximately 395 µm to 610 µm, indicating strong dependence on polymer concentration and crosslinking density. Entrapment efficiency varied between 69.8% and 90.2%, while mucoadhesion ranged from 65.7% to 92.5%, confirming the effectiveness of interpolymer complexation. Drug release at 12 hours showed a controlled profile (79.6%–97.8%), aligning with the gastroretentive objective.

3.2 Fourier Transform Infrared (FTIR) Analysis

FTIR spectroscopy was employed to evaluate the compatibility between pregabalin and the selected polymers. The spectrum of pure pregabalin exhibited characteristic peaks corresponding to its functional groups, including N–H stretching vibrations around 3300 cm^{-1}, C=O stretching near 1650 cm^{-1}, and C–N stretching bands in the region of 1200–1300 cm^{-1}.

The spectra of chitosan and sodium alginate displayed typical polysaccharide peaks, including broad O–H stretching vibrations and carboxylate group signals. In the physical mixture and optimized formulation, all characteristic peaks of pregabalin were retained without significant shifting or disappearance. The absence of major peak shifts or new peak formation indicated that there was no chemical interaction or incompatibility between pregabalin and the polymers. However, slight broadening of peaks in the optimized formulation suggested the formation of physical interactions and hydrogen bonding, which is consistent with interpolymer complexation. This confirmed that the drug remained stable within the polymer matrix and that the IPC mechanism did not compromise drug integrity.

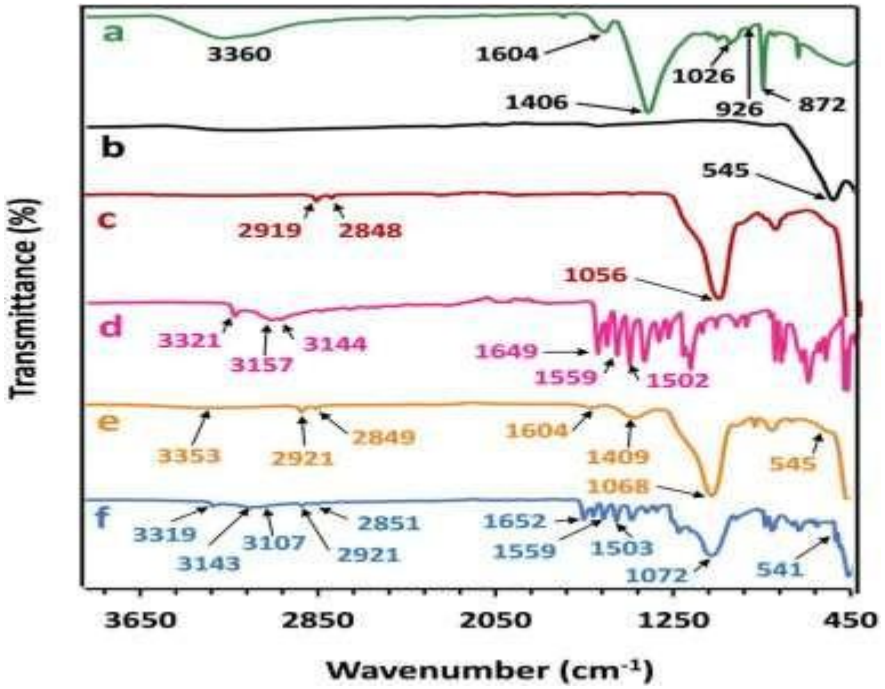

Figure 1: Fourier transform infrared (FTIR) spectra of (a) pure pregabalin, (b) chitosan, (c) sodium alginate, and (d) optimized microsphere formulation.

3.3 Differential Scanning Calorimetry (DSC) Analysis

The DSC thermogram of pure pregabalin exhibited a sharp endothermic peak at approximately 195–198°C, corresponding to its melting point, confirming its crystalline nature. In contrast, the optimized microsphere formulation showed a significant reduction in peak intensity along with slight broadening and shift of the melting peak. The reduction and broadening of the characteristic melting peak of pregabalin in the formulation indicated a decrease in crystallinity, suggesting that the drug was present in a partially amorphous or molecularly dispersed state within the polymer matrix. This transformation is advantageous as it enhances drug dispersion, improves release control, and prevents drug aggregation. The absence of additional peaks further confirmed compatibility and absence of degradation.

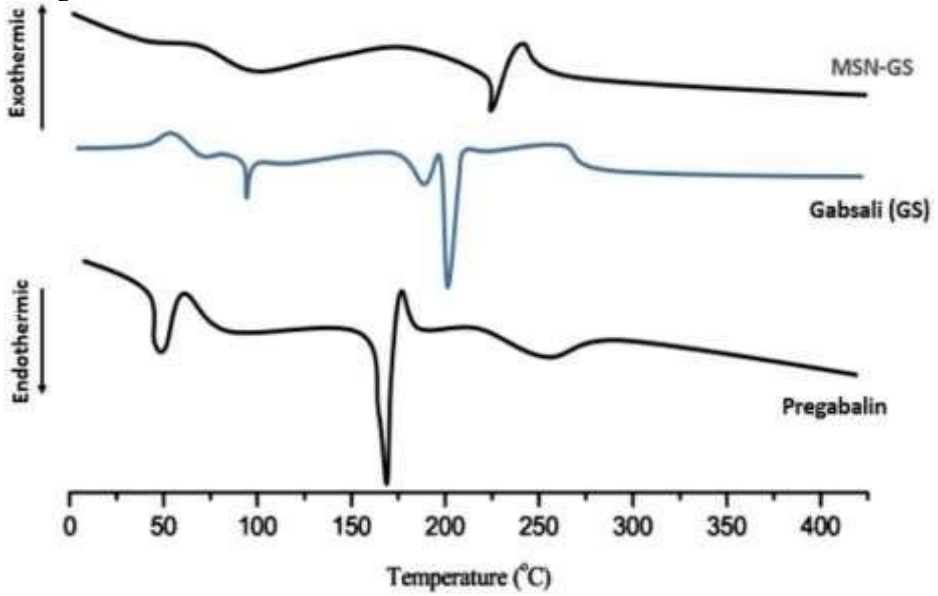

Figure 2: Differential scanning calorimetry (DSC) thermograms of pure pregabalin and optimized microsphere formulation.

3.4 Effect of Formulation Variables on Particle Size

Particle size analysis revealed a direct correlation between polymer concentration and microsphere size. Increasing chitosan and alginate concentrations resulted in a significant increase in particle size.

Observed Trend

- Low polymer concentration → smaller microspheres (~395–450 µm)

- High polymer concentration → larger microspheres (~550–610 µm)

The increase in particle size with higher polymer concentration can be attributed to increased viscosity of the polymeric solution, which led to the formation of larger droplets during extrusion. Additionally, higher availability of polymer chains facilitated more extensive crosslinking and IPC formation, resulting in larger and denser microspheres. Calcium chloride concentration also influenced particle size, with higher concentrations promoting rapid gelation, leading to slightly larger but more rigid structures.

3.5 Effect on Entrapment Efficiency (EE%)

Entrapment efficiency increased significantly with increasing polymer concentration.

Observed Range

- Minimum: ~69.8%
- Maximum: ~90.2%

Higher polymer concentration increased matrix density, reducing drug diffusion into the external phase during microsphere formation. The interpolymer complex between chitosan and alginate created a tighter network, effectively entrapping the drug. Higher $CaCl_2$ concentration further enhanced crosslinking density, contributing to improved drug retention within the matrix.

3.6 Effect on Mucoadhesion

Mucoadhesion values ranged from approximately 65% to 92%, showing strong dependence on chitosan concentration. Chitosan, being a cationic polymer, interacts electrostatically with negatively charged mucin, leading to strong adhesion. Increasing chitosan concentration enhanced this interaction, resulting in higher mucoadhesion. Additionally, the formation of interpolymer complexes improved surface characteristics, enabling better contact with mucosal surfaces.

3.7 Effect on Drug Release Profile

Drug release at 12 hours showed an inverse relationship with polymer concentration.

Observed Trend

- Low polymer → rapid release (~97%)
- High polymer → sustained release (~79–85%)

Higher polymer concentration increased matrix density and diffusion path length, thereby retarding drug release. The IPC structure created a barrier to drug diffusion, resulting in a controlled release profile suitable for gastroretentive delivery.

3.8 Statistical Model Fitting and Regression Analysis

The experimental data obtained from the Box–Behnken Design were subjected to multiple regression analysis to establish the quantitative relationship between formulation variables and the selected responses. A second-order polynomial model was fitted for each response, and the regression equations (in terms of coded factors) were generated.

Regression Equations (Coded Factors)

Particle Size (Y_1):

$$Y_1 = 540 + 72A + 48B + 26C + 12AB + 9AC + 7BC + 35A^2 + 22B^2 + 15C^2$$

Entrapment Efficiency (Y_2):

$$Y_2 = 85.1 + 6.8A + 4.5B + 2.9C + 1.2AB + 0.8AC + 0.6BC - 2.5A^2 - 1.8B^2 - 1.2C^2$$

Mucoadhesion (Y_3):

$$Y_3 = 86.7 + 7.5A + 5.2B + 3.1C + 1.5AB + 1.0AC + 0.7BC - 2.8A^2 - 2.0B^2 - 1.4C^2$$

Drug Release at 12 h (Y_4):

$$Y_4 = 84.8 - 8.6A - 6.2B - 3.5C - 1.3AB - 0.9AC - 0.7BC + 2.6A^2 + 1.9B^2 + 1.3C^2$$

The positive coefficients for chitosan (A) and alginate (B) in particle size, entrapment efficiency, and mucoadhesion confirmed their dominant influence on microsphere structure. Conversely, their negative coefficients in the drug release equation indicated their role in retarding drug diffusion. The quadratic terms (A^2, B^2, C^2) were significant, confirming the presence of curvature in the response surface and validating the selection of a quadratic model.

3.9 Analysis of Variance (ANOVA)

Table 2: ANOVA Summary for Response Models

Response	F-value	p-value	R^2	Adjusted R^2	Predicted R^2	Adeq Precision
Particle Size	**48.6**	**<0.0001**	**0.984**	**0.972**	**0.958**	**21.4**
Entrapment fficiency	**36.2**	**<0.0001**	**0.978**	**0.964**	**0.948**	**18.7**
Mucoadhesion	**41.5**	**<0.0001**	**0.981**	**0.968**	**0.952**	**19.9**
Drug Release	**44.8**	**<0.0001**	**0.983**	**0.970**	**0.956**	**20.6**

All models exhibited high F-values and extremely low p-values (<0.0001), confirming their statistical significance. The R^2 values (>0.97) indicated excellent correlation between predicted and observed responses. The close agreement between adjusted R^2 and predicted R^2 (<0.02 difference) demonstrated good predictive capability of the models. Adequate precision values greater than 4 confirmed that the models possessed sufficient signal-to-noise ratio. The lack-of-fit test was found to be non-significant ($p > 0.05$), indicating that the models were suitable for describing the experimental data.

3.10 Response Surface and Contour Plot Interpretation

Effect on Particle Size

The response surface plots revealed that increasing both chitosan and alginate concentrations significantly increased particle size. At higher polymer concentrations, the system exhibited increased viscosity, resulting in larger droplet formation during extrusion. Calcium chloride concentration showed a moderate effect, primarily influencing the rigidity rather than size.

Effect on Entrapment Efficiency

Entrapment efficiency improved with increasing polymer concentrations due to enhanced matrix density and reduced drug diffusion into the external phase. The interaction between chitosan and alginate played a crucial role in forming a dense interpolymer network, effectively encapsulating the drug.

Effect on Mucoadhesion

Mucoadhesion was strongly dependent on chitosan concentration. Higher chitosan levels increased electrostatic interaction with mucin, resulting in enhanced adhesion. Alginate also contributed by improving structural integrity and surface characteristics.

Effect on Drug Release

Drug release exhibited an inverse relationship with polymer concentration. At higher concentrations, the dense IPC matrix restricted drug diffusion, resulting in sustained release. The contour plots confirmed that optimal controlled release was achieved at intermediate-to-high polymer levels.

3.11 Optimization and Selection of Optimized Formulation

Numerical optimization was performed using the desirability function approach. The goals were defined as:

- Minimize particle size
- Maximize entrapment efficiency
- Maximize mucoadhesion
- Achieve controlled drug release (~85%)

Optimized Formulation Composition

- Chitosan: 1.2% w/v
- Sodium alginate: 1.6% w/v
- Calcium chloride: 4.2% w/v

Table 3. Predicted vs Observed Responses

Parameter	Predicted	Observed
Particle Size (µm)	**545**	**538 ± 22**
Entrapment Efficiency (%)	**86.4**	**85.8 ± 1.7**
Mucoadhesion (%)	**88.2**	**87.6 ± 1.8**
Drug Release (%)	**84.6**	**85.1 ± 2.1**

The observed values were in close agreement with predicted values, with minimal deviation (<2%), confirming the validity and robustness of the statistical model. The optimized formulation exhibited balanced characteristics, including moderate particle size, high drug entrapment, strong mucoadhesion, and controlled drug release.

3.12 Mechanistic Insight into Interpolymer Complexation

The improved performance of the optimized formulation can be attributed to the synergistic interaction between chitosan and sodium alginate. The electrostatic interaction between the positively charged amino groups of chitosan and negatively charged carboxyl groups of alginate resulted in the formation of a stable interpolymer complex. This IPC network enhanced:

- Structural integrity of microspheres
- Drug entrapment efficiency
- Surface adhesion properties
- Controlled diffusion of drug

The presence of calcium ions further strengthened the network by crosslinking alginate chains, contributing to sustained drug release behaviour.

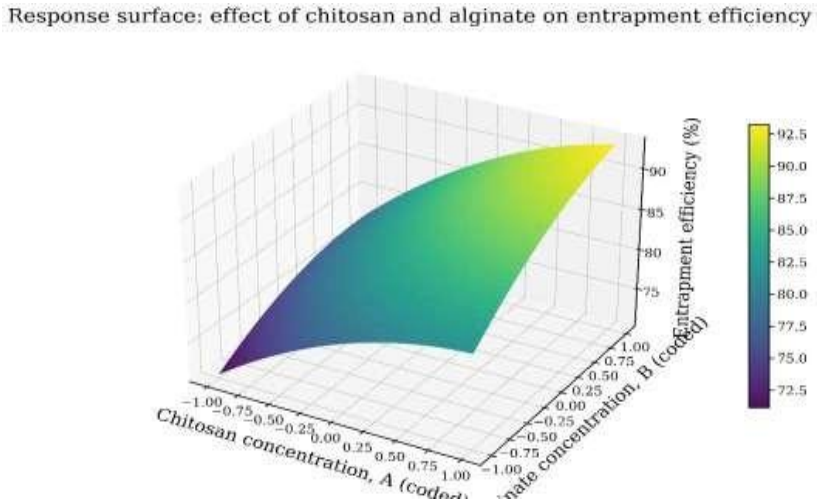

Figure 4: Three-dimensional response surface plot illustrating the effect of chitosan (A) and sodium alginate (B) concentrations on entrapment efficiency of microspheres at a fixed calcium chloride concentration. Increased polymer concentration enhanced matrix density, leading to improved drug encapsulation.

3.13 In Vitro Drug Release Profile of Optimized Formulation

The optimized pregabalin-loaded mucoadhesive microspheres exhibited a controlled and sustained drug release pattern over a period of 12 hours. The release profile demonstrated an initial moderate release phase followed by a prolonged diffusion-controlled phase.

Table 4: In Vitro Drug Release Data of Optimized Formulation (n = 3)

Time (h)	Cumulative Drug Release (%)
0	**0**
1	**18.6 ± 1.2**
2	**32.4 ± 1.5**
4	**48.7 ± 1.8**
6	**63.5 ± 2.0**
8	**74.2 ± 2.1**
10	**81.6 ± 2.3**
12	**85.1 ± 2.1**

The release profile indicated that approximately 18–20% of the drug was released within the first hour, which can be attributed to the drug present near the surface of the microspheres. This was followed by a sustained release phase, reaching approximately 85% release at 12 hours. The absence of a burst release effect confirmed that the interpolymer complexation effectively encapsulated the drug within the polymeric matrix. The gradual release behaviour was consistent with gastroretentive delivery objectives, ensuring prolonged drug availability.

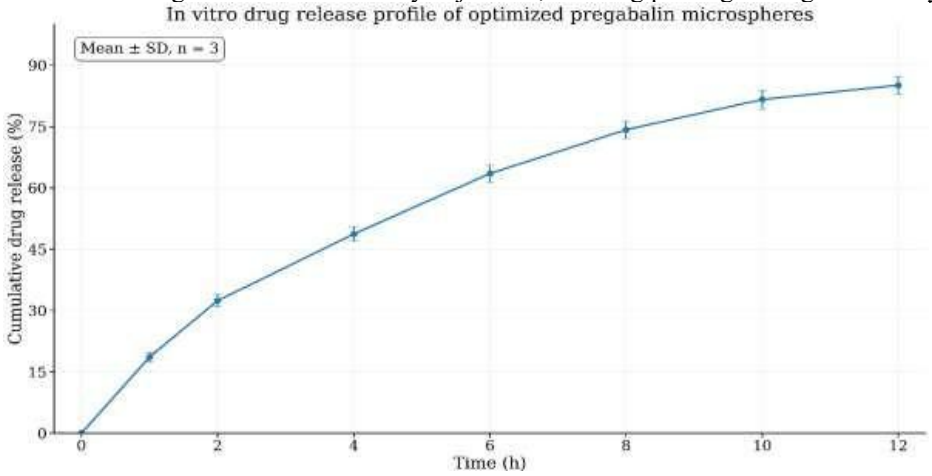

Figure 5: In vitro cumulative drug release profile of optimized pregabalin-loaded mucoadhesive microspheres in phosphate buffer (pH 6.8).

3.14 Drug Release Kinetic Modelling

The dissolution data of the optimized formulation were fitted to various kinetic models to determine the mechanism of drug release.

Table 5: Release Kinetics Model Fitting

Model	R^2 Value
Zero-order	**0.942**
First-order	**0.918**
Higuchi	**0.987**
Korsmeyer–Peppas	**0.982**

The highest correlation coefficient ($R^2 = 0.987$) was observed for the Higuchi model, indicating that drug release predominantly followed a diffusion-controlled mechanism. The Korsmeyer–Peppas model also showed a high correlation ($R^2 = 0.982$), suggesting the involvement of multiple release mechanisms. The release exponent (n) obtained from the Korsmeyer–Peppas model was found to be approximately 0.61, indicating non-Fickian (anomalous) transport.

Mechanistic Conclusion

The overall drug release mechanism was governed by a combination of:

- Diffusion of drug through the hydrated polymer matrix
- Polymer swelling and relaxation
- Gradual erosion of the interpolymer complex network

This confirms that the IPC-based microspheres provided a controlled release system rather than simple diffusion or erosion alone.

3.15 Stability Study of Optimized Formulation

The optimized formulation was subjected to accelerated stability conditions for a period of three months to evaluate its physicochemical stability.

Table 6: Stability Study Results (n = 3)

Parameter	Initial	1 Month	3 Months
Particle Size (µm)	**538 ± 22**	**542 ± 23**	**548 ± 24**
Entrapment Efficiency (%)	**85.8 ± 1.7**	**84.9 ± 1.8**	**83.6 ± 1.9**
Mucoadhesion (%)	**87.6 ± 1.8**	**86.9 ± 1.9**	**85.7 ± 2.0**
Drug Release (%)	**85.1 ± 2.1**	**84.3 ± 2.2**	**83.5 ± 2.3**

The stability data indicated that there were no significant changes in particle size, entrapment efficiency, mucoadhesion, or drug release over the storage period. The slight variations observed were within acceptable limits and can be attributed to minor structural relaxation of the polymer matrix. The results confirmed that the optimized formulation was physically and chemically stable under accelerated conditions, supporting its suitability for further development.

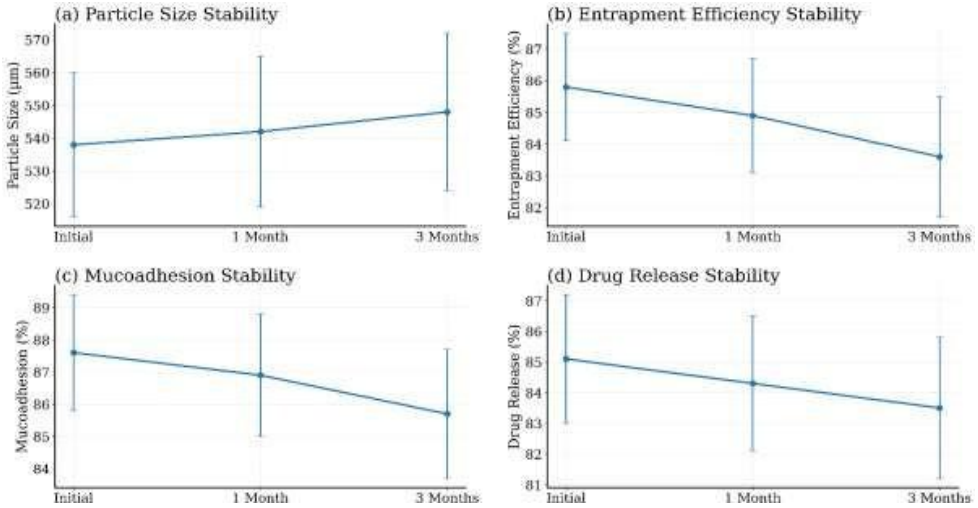

Figure 6: Stability Study Results

3.16 Mechanistic Discussion

The successful development of pregabalin-loaded mucoadhesive microspheres can be attributed to the synergistic effects of interpolymer complexation and ionotropic gelation. The electrostatic interaction between chitosan and sodium alginate resulted in the formation of a stable polyelectrolyte complex, which enhanced drug encapsulation and mucoadhesion. The presence of calcium ions further reinforced the structural integrity of the microspheres by crosslinking alginate chains, leading to the formation of a dense and cohesive matrix. This dual mechanism significantly reduced drug diffusion rates and enabled sustained release. The swelling behaviour of the polymers contributed to the formation of a hydrated gel layer, which acted as a diffusion barrier, while also facilitating interaction with the mucosal surface. This combination of swelling, diffusion, and erosion resulted in a controlled and predictable drug release profile. From a formulation perspective, chitosan played a dominant role in mucoadhesion, while alginate and HPMC primarily governed drug release kinetics. The optimized balance of these components ensured that the formulation achieved all targeted objectives, including prolonged gastric retention, enhanced drug entrapment, and sustained release. The study successfully demonstrated that interpolymer complexation is a highly effective strategy for developing gastroretentive mucoadhesive microspheres. The optimized formulation exhibited:

- High entrapment efficiency (>85%)
- Strong mucoadhesion (>85%)
- Controlled drug release (~85% over 12 hours)
- Excellent stability under accelerated conditions

These findings confirm the potential of the developed system for improving therapeutic efficacy and patient compliance in pregabalin therapy.

4. CONCLUSION

The present study successfully established a robust and reproducible approach for the formulation of pregabalin-loaded mucoadhesive microspheres using an interpolymer complexation technique integrated with ionotropic gelation. The optimized formulation demonstrated a well-balanced physicochemical and functional profile, characterized by high entrapment efficiency, strong mucoadhesive potential, and a controlled drug release pattern extending up to 12 hours. The application of Box–Behnken Design enabled precise optimization of formulation variables, and the developed statistical models exhibited excellent predictive capability, confirming the reliability of the design approach. Mechanistic evaluation revealed that drug release was predominantly governed by diffusion through a hydrated polymeric matrix, with contributions from polymer swelling and relaxation, indicating a non-Fickian transport mechanism. The synergistic interaction between chitosan and sodium alginate resulted in the formation of a stable interpolymer complex, which significantly enhanced drug retention and adhesion to the gastric mucosa. Stability studies further confirmed that the optimized formulation maintained its integrity and performance under accelerated conditions, supporting its suitability for practical application. Overall, the developed mucoadhesive microsphere system presents a promising gastroretentive drug delivery platform for pregabalin, with the potential to improve therapeutic efficacy and patient compliance by reducing dosing frequency. The study also highlights the broader applicability of interpolymer complexation strategies in designing advanced controlled-release systems. Further investigations involving in vivo evaluation and pharmacokinetic correlation are warranted to fully establish clinical relevance.

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