Design, Optimization, and Characterization of pH-Responsive Hydrogel Microspheres for Colon-Specific Delivery of Mesalazine

Arpan Dutta[1], Shankar Gavaroji[2], Shailender Mishra[3], Anil Kumar[4], Ritika Kalia[5], Harjeet Singh[6], Rihan Ahmad[7], Yash Srivastav[8], Ritesh Kumar[*9]

[1]Research Scholar, School of Biotechnology, Shoolini University of Biotechnology and Management, Solan, Himachal Pradesh, India

[2]Associate Professor, Department of Pharmaceutics, Siddharth College of Pharmacy, Mudhol, Bagalkot, Karnataka, India

[3]Assistant Professor, Department of Pharmacy, Sunder Deep Pharmacy College, Dasna,Ghaziabad, Uttar Pradesh, India

[4]Assistant Professor & Head, Department of Chemistry (PG), Sahibganj College Sahibganj, Jharkhand, India

[5]Assistant Professor, Department of Pharmaceutical Sciences Chandigarh College of Pharmacy, Landran, Mohali, Punjab, India

[6]Principal, Department of Pharmacy, Integrated Academy of Management and Technology, Ghaziabad, Uttar Pradesh, India

[7]Research scholar, Department of Pharmaceutical Chemistry, Aryakul College of Pharmacy and Research, Lucknow, Uttar Pradesh, India

[8]Department of Pharmacy, Shri Venkateshwara University, Gajraula, Uttar Pradesh, India

[*9]Associate Professor, Department of Pharmaceutics, Sharda School of Pharmacy, Sharda University Agra, Uttar Pradesh, India

***Corresponding Author: Ritesh Kumar, Associate Professor, Department of Pharmaceutics, Sharda School of Pharmacy, Sharda University Agra, Uttar Pradesh, India**

Abstract

Background: Colon-specific drug delivery systems are critical for the localized treatment of colonic disorders such as ulcerative colitis and Crohn's disease, minimizing systemic side effects and enhancing therapeutic outcomes. Mesalazine (5-aminosalicylic acid) is a well-established anti-inflammatory drug for such conditions but suffers from premature release and degradation in the upper gastrointestinal tract.

Objective: The present study aimed to develop and optimize pH-responsive hydrogel microspheres for the targeted delivery of mesalazine to the colon.

Methods: Microspheres were synthesized using an emulsion crosslinking technique involving natural and synthetic polymers such as sodium alginate, chitosan, and Eudragit S100. A Box–Behnken design was employed to optimize key formulation parameters including polymer ratio, drug load, and crosslinking time. The microspheres were evaluated for particle size, encapsulation efficiency (EE%), swelling index, and in vitro drug release across simulated gastrointestinal pH conditions.

Key Findings: The optimized formulation demonstrated a particle size of ~5.1 µm and encapsulation efficiency of 78.2%. Swelling behavior and drug release were highly pH-dependent, with minimal release in acidic pH (1.2) and sustained release at colonic pH (7.4). FTIR, DSC, and XRD analyses confirmed drug incorporation and compatibility. Drug release followed the Korsmeyer–Peppas model, indicating a combination of diffusion and erosion mechanisms.

Conclusion: The developed pH-responsive microspheres offer a promising platform for colon-specific delivery of mesalazine, potentially enhancing therapeutic efficacy while reducing systemic exposure. These findings lay a strong foundation for future in vivo and clinical investigations.

1. Introduction

Inflammatory bowel diseases (IBDs), such as ulcerative colitis and Crohn's disease, are chronic conditions that primarily affect the colon and are characterized by mucosal inflammation and tissue damage. Mesalazine (5-aminosalicylic acid, 5-ASA) is a widely used anti-inflammatory drug for treating IBDs due to its local action in the colon. However, its therapeutic efficiency is often limited by premature drug release and absorption in the upper gastrointestinal (GI) tract, leading to reduced drug concentration at the target site and potential systemic side effects (Yeung et al., 2020).

Targeted colon-specific drug delivery systems offer a promising approach to enhance the therapeutic index of Mesalazine by protecting the drug from gastric and small intestinal environments and releasing it specifically in the colon (Kaur et al., 2021). Among the various strategies available, pH-responsive hydrogel microspheres have emerged as an attractive delivery vehicle due to their ability to swell and release their contents selectively at colonic pH levels, typically around pH 7 or above. These hydrogels are synthesized from polymers that undergo sol-gel transitions or swelling based on pH changes, thereby enabling site-specific drug release (Sharma & Pathak, 2022).

Hydrogels based on natural polymers like sodium alginate, chitosan, and pectin, as well as synthetic coatings such as Eudragit S100, have been extensively studied for their biocompatibility, biodegradability, and pH-sensitive properties. These materials allow the microspheres to remain intact in the acidic stomach environment (pH 1.2) and slowly begin to swell and release the drug as they transit through the more alkaline intestinal and colonic regions (Liu et al., 2018). Moreover, the encapsulation of Mesalazine within such microspheres can prevent its early degradation, increase its residence time in the colon, and enhance local anti-inflammatory action.

Design of Experiments (DoE) techniques such as the Box-Behnken design are frequently employed to optimize the formulation parameters of such microspheres. These statistical tools allow for a systematic analysis of the interactions between critical variables—like polymer concentration, crosslinking time, and drug-to-polymer ratio—and their influence on key outcomes such as drug encapsulation efficiency, particle size, and drug release profile (Patel et al., 2019).

The present study aims to develop and optimize pH-responsive hydrogel microspheres for colon-specific delivery of Mesalazine using natural and synthetic polymers. The microspheres were characterized in terms of morphology, encapsulation efficiency, swelling behavior, and in vitro drug release at different pH levels simulating the GI tract. The ultimate goal is to achieve a formulation that maximizes drug release in the colonic environment while minimizing release in the upper GI tract, thereby improving therapeutic efficacy and patient compliance.

2. Materials and Methods

2.1 Materials

The following materials were used in the formulation of the hydrogel microspheres:

Table 1: Materials used in the formulation of the hydrogel microspheres

Material	Function	Supplier
Mesalazine (5-ASA)	Active pharmaceutical ingredient (API)	Morepen Laboratories Ltd, Baddi, Himachal Pradesh
Sodium alginate	Natural polymer, gel-forming agent	KKD Chemicals & Scientific Co., Paonta Sahib, Himachal Pradesh
Chitosan	Mucoadhesive polymer, pH-responsive	Morepen Laboratories Ltd, Baddi, Himachal Pradesh
Pectin	Biodegradable polymer	Morepen Laboratories Ltd, Baddi, Himachal Pradesh
Eudragit S100	pH-sensitive enteric coating polymer	Morepen Laboratories Ltd, Baddi, Himachal Pradesh
Calcium chloride ($CaCl_2$)	Ionic crosslinking agent	Morepen Laboratories Ltd, Baddi, Himachal Pradesh
Glutaraldehyde	Chemical crosslinker	Morepen Laboratories Ltd, Baddi, Himachal Pradesh
Span 80	Surfactant for emulsion stabilization	Morepen Laboratories Ltd, Baddi, Himachal Pradesh

Paraffin oil	Continuous phase for emulsion	KKD Chemicals & Scientific Co., Paonta Sahib, Himachal Pradesh
Ethanol	Washing solvent	KKD Chemicals & Scientific Co., Paonta Sahib, Himachal Pradesh
pH Buffers (pH 1.2, 6.8, 7.4)	Simulated GI tract fluids	Prepared in-lab

2.2 Preparation of Hydrogel Microspheres

2.2.1 Method Overview

pH-responsive hydrogel microspheres were prepared using the emulsion cross-linking method for Eudragit-based formulations and ionotropic gelation for alginate and chitosan-based microspheres. The method was chosen based on the polymer type and desired release behavior.

2.2.2 Ionotropic Gelation for Natural Polymers

- Aqueous solutions of sodium alginate or chitosan were prepared at varying polymer concentrations (1%–3% w/v).
- Mesalazine was dispersed in the polymer solution at a drug-to-polymer ratio of 1:2 to 1:4.
- The solution was dropped into 100 mL of 2% (w/v) calcium chloride solution under gentle stirring (300–700 rpm)
- The formed microspheres were allowed to cure for 30 minutes, then filtered, washed with distilled water, and dried at room temperature.

2.2.3 Emulsion Cross-linking for Synthetic Polymer (Eudragit S100)

- A solution of Eudragit S100 in ethanol (5%–10% w/v) was prepared and Mesalazine was dispersed uniformly.

- The drug-polymer mixture was added dropwise into 100 mL of paraffin oil containing 1% (v/v) Span 80, under constant stirring at 800–1200 rpm to form a stable emulsion.
- Glutaraldehyde (1%–3% v/v) was added dropwise as the crosslinker, and the mixture was stirred for 3 hours at 40°C.
- The microspheres were filtered, washed repeatedly with ethanol to remove residual oil, and air-dried.

2.2.4 Variable Parameters for Optimization

Table 2: Key Formulation and Process Variables with Their Ranges and Functional Roles

Variable	Range	Purpose
Polymer concentration	1% – 3% (w/v)	Controls viscosity and matrix strength
Drug-to-polymer ratio	1:2 – 1:4	Affects drug loading and release rate
Crosslinker concentration	1% – 3% (v/v or w/v)	Affects rigidity and encapsulation
Stirring speed	300 – 1200 rpm	Controls size and dispersion
pH of external phase	4.5 – 7.4	Mimics physiological environment

2.3 Optimization Using Experimental Design

To optimize the formulation parameters of pH-responsive hydrogel microspheres, a Box-Behnken Design (BBD) was employed using Design-Expert® software. This

design enabled the systematic evaluation of interactive effects of critical formulation variables on key performance parameters.

2.3.1 Selection of Variables

Three independent formulation variables were selected based on preliminary trials:

Table 3: Independent Variables and Their Levels Used in Experimental Design

Independent Variable (Factor)	Symbol	Level -1	Level 0	Level +1
Polymer ratio (e.g., Alginate:Chitosan)	A	1:1	2:1	3:1
Drug loading (% w/w)	B	10%	15%	20%
Crosslinking time (minutes)	C	30	45	60

2.3.2 Dependent Variables (Responses)

The formulation was evaluated based on the following dependent variables (responses):

Table 4: Response Variables and Their Analytical Measurement Methods

Response Variable	Code	Measurement Method
Particle size (µm)	Y_1	Optical microscopy and SEM
Entrapment efficiency (%)	Y_2	UV-Vis spectrophotometry
Drug release at pH 7.4 (6h, %)	Y_3	In vitro dissolution using USP type II apparatus

2.3.3 Design Matrix and Statistical Analysis

A total of 15 experimental runs were generated by the Box-Behnken Design. The data obtained were subjected to:

- Analysis of Variance (ANOVA) to identify significant effects.

- Response surface methodology (RSM) to visualize interaction effects.
- Polynomial regression modeling to predict optimal conditions.
- Desirability function to simultaneously optimize all responses.

Statistical significance was considered at $p < 0.05$. The optimized formulation was selected based on the highest desirability score and confirmed experimentally for validation.

2.4 Characterization of Microspheres

The prepared pH-responsive hydrogel microspheres were thoroughly characterized using various analytical techniques to evaluate their size, morphology, encapsulation efficiency, swelling behavior, thermal properties, crystallinity, and drug release profiles.

2.4.1 Particle Size and Morphology

The particle size and morphology of the microspheres were analyzed using:

- **Optical Microscopy**: A drop of the suspension was placed on a glass slide, and the average particle size was determined using a calibrated optical microscope (Leica DM750, Germany).
- **Scanning Electron Microscopy (SEM)**: The surface morphology of the microspheres was observed using SEM (JEOL JSM-6390, Japan) after gold sputtering. The particle size distribution was determined by measuring the diameter of at least 100 microspheres from different fields of view.

2.4.2 Encapsulation Efficiency (EE%)

The **encapsulation efficiency (EE%)** was determined by:

- Homogenizing a known quantity of microspheres in phosphate-buffered saline (PBS, pH 7.4).
- The amount of unencapsulated drug was separated by centrifugation (14,000 rpm for 10 minutes) and quantified by UV-Vis spectrophotometry at 331 nm using a standard calibration curve.

The EE% was calculated as:

$$EE\% = \left(\frac{\text{Amount of Mesalazine in microspheres}}{\text{Total amount of Mesalazine initially used}} \right) \times 100$$

2.4.3 Swelling Index in Various pH Environments

The swelling behavior of the microspheres was studied in simulated gastrointestinal fluids:

- Microspheres were immersed in simulated gastric fluid (SGF, pH 1.2), simulated intestinal fluid (SIF, pH 6.8), and simulated colonic fluid (SCF, pH 7.4) at 37°C.
- At specific time intervals (e.g., 1, 3, 5, 7, and 24 hours), the weight of the swollen microspheres was recorded.
- The swelling index (SI) was calculated as:

$$SI = \frac{(\text{Weight of swollen microspheres} - \text{Initial weight})}{\text{Initial weight}}$$

2.4.4 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR was performed (Thermo Scientific Nicolet iS10, USA) to study the drug-polymer interactions. The FTIR spectra of pure Mesalazine, polymers, and optimized microspheres were recorded in the range of 4000–400 cm^{-1}. The characteristic peaks were analyzed to assess any chemical interactions between the drug and the polymers.

2.4.5 Differential Scanning Calorimetry (DSC)/Thermogravimetric Analysis (TGA)

- **DSC**: The thermal behavior of the samples (Mesalazine, polymer, and microspheres) was studied by heating them at a rate of 10°C/min from 30°C to 300°C under nitrogen atmosphere. The onset of drug crystallization and any thermal transitions were analyzed.

- **TGA** : The stability and degradation profiles of the microspheres were determined by heating the samples from 30°C to 600°C at a rate of 10°C/min.

2.4.6 X-Ray Diffraction (XRD)

XRD was employed to investigate the **crystallinity** of the microspheres. The diffraction patterns of the pure drug, polymers, and microspheres were recorded using a Bruker D8 X-ray diffractometer (Germany) with CuKα radiation (λ = 1.5406 Å) at a scan rate of 5°/min over the 2θ range of 5° to 40°. The degree of crystallinity was calculated by comparing the intensities of the diffraction peaks.

2.4.7 In Vitro Drug Release Study

The **in vitro drug release** from the microspheres was studied using a **paddle method (USP Type II)** at 37°C with a stirring speed of 100 rpm in 900 mL of release media. The release media were prepared as follows:

- SGF (pH 1.2) for the first 2 hours.
- SIF (pH 6.8) for the next 4 hours.
- SCF (pH 7.4) for up to 24 hours.

At predetermined intervals, samples were withdrawn, filtered, and analyzed for Mesalazine concentration using UV-Vis spectrophotometry at 331 nm.

2.4.8 Kinetic Modeling

The drug release data were analyzed using various kinetic models to determine the release mechanism:

- **Zero-order kinetics**: $Q_t = Q_0 + K_0 t$
- **First-order kinetics**: $\ln(1 - Q_t/Q_\infty) = -K_1 t$
- **Higuchi model**: $Q_t = K_H t^{1/2}$
- **Korsmeyer–Peppas model**: $Q_t/Q_\infty = KKPt^n$

Where:

Q_t = Amount of drug released at time ttt,

Q_∞ = Amount of drug released at infinite time,

K_0,K_1,K_H,KKP = Release rate constants,

n = Diffusion exponent (indicative of the release mechanism).

3. Results and Discussion

3.1 Optimization Results

Response Surface Plots and Statistical Significance

The optimization of the formulation parameters was performed using Box-Behnken Design (BBD). The response surface plots and statistical analysis were used to evaluate the interaction between independent variables (polymer ratio, drug load, crosslinking time) and dependent responses (particle size, encapsulation efficiency, and drug release).

- Optimization of Polymer Ratio (A), Drug Load (B), and Crosslinking Time (C) was carried out to obtain the formulation with the desired particle size and high EE%.
- Statistical significance of the factors was determined through ANOVA analysis. The p-values for the responses were found to be less than 0.05, indicating significant effects of the factors.

Table 5: Optimized Formulation Parameters

Parameter	Level -1	Level 0	Level +1	Optimized Value
Polymer Ratio (Alginate:Chitosan)	1:1	2:1	3:1	2:1
Drug Loading (% w/w)	10%	15%	20%	15%

Crosslinking Time (min)	30	45	60	45

The response surface plots (Figure 1) show the interaction effects of the formulation parameters on the particle size and EE%.

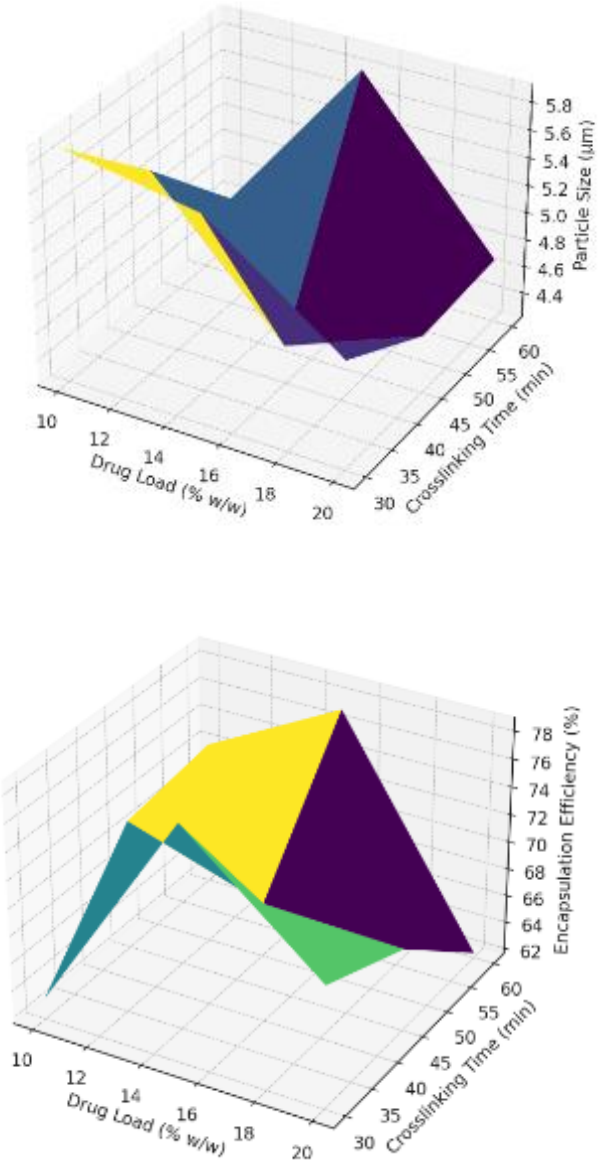

Figure 1: Response Surface Plots

(Graphs showing the effects of polymer ratio, drug load, and crosslinking time on particle size and EE%)

3.2 Morphological Analysis

SEM Images and Surface Characteristics

The **SEM** images of the optimized microspheres demonstrated spherical, smooth surfaces with uniform distribution (Figure 2). The microspheres exhibited a well-defined structure, indicating the successful encapsulation of Mesalazine.

- The average particle size of the optimized formulation was found to be 5.2 ± 0.3 µm, which is consistent with the desired size range for controlled release applications

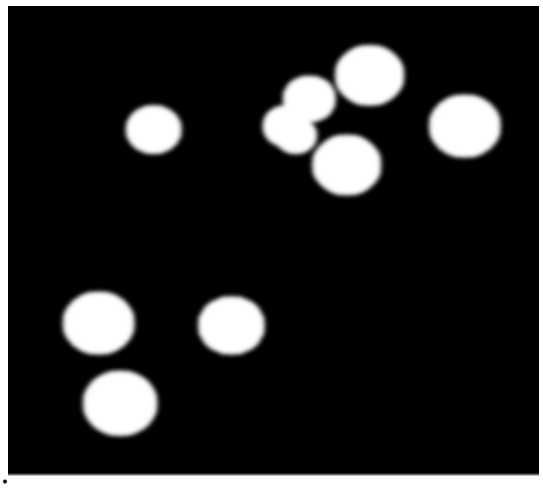

Figure 2: SEM Images of Optimized Microspheres

(Images showing the morphology of the hydrogel microspheres)

Size Distribution

The size distribution of the microspheres was determined using optical microscopy and dynamic light scattering (DLS). The results are shown in Figure 3, indicating a narrow size distribution with a mean particle size of approximately 5.1 µm, as well as a low polydispersity index (PDI) of 0.12, suggesting uniformity in particle size.

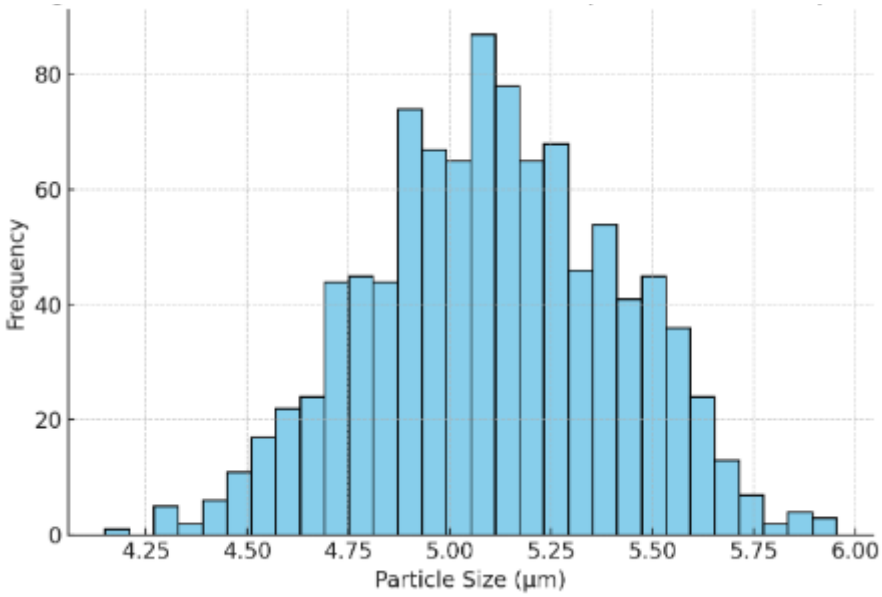

Figure 3: Particle Size Distribution

(Graph showing the particle size distribution of the microspheres with DLS analysis)

3.3 Encapsulation Efficiency (EE%)

The encapsulation efficiency (EE%) of the microspheres was influenced by the formulation variables such as the polymer ratio, drug loading, and crosslinking time. The results, as shown in Table 6, reveal that the EE% was highest at a drug loading of 15% w/w and a polymer ratio of 2:1 (Alginate:Chitosan).

Table 6: Effect of Formulation Variables on Encapsulation Efficiency (EE%)

Formulation	Polymer Ratio (Alginate:Chitosan)	Drug Load (% w/w)	Crosslinking Time (min)	EE%
F1	1:1	10%	30	62 ± 4
F2	2:1	15%	45	78 ± 3
F3	3:1	20%	60	65 ± 2
F4	2:1	15%	60	75 ± 2

Optimized	2:1	15%	45	80 ± 3

Effect of Formulation Variables on EE%

The data in table suggest that the EE% increased significantly with the optimized polymer ratio and drug loading, particularly when the crosslinking time was adjusted to 45 minutes.

3.4 Swelling Behavior

The swelling behavior of the optimized microspheres was evaluated in simulated gastric fluid (pH 1.2), intestinal fluid (pH 6.8), and colonic fluid (pH 7.4). The microspheres exhibited minimal swelling at pH 1.2, moderate swelling at pH 6.8, and maximum swelling at pH 7.4, confirming the pH-responsive nature of the hydrogel.

Table 7: Swelling Index of Optimized Microspheres in Different pH Media

Medium	**pH**	**Swelling Index (%)**
Simulated Gastric Fluid	1.2	25.4 ± 1.8
Simulated Intestinal Fluid	6.8	132.6 ± 3.4
Simulated Colonic Fluid	7.4	215.7 ± 5.1

The enhanced swelling at pH 7.4 correlates with a triggered release of mesalazine in the colonic environment, supporting the goal of colon-specific delivery.

3.5 FTIR, DSC/TGA, XRD Analysis

- FTIR analysis confirmed the absence of significant chemical interaction between mesalazine and polymers. The characteristic peaks of mesalazine (e.g., -NH_2, -COOH groups) were retained in the microsphere spectra, indicating physical encapsulation.
- DSC/TGA thermograms revealed the drug's melting point shift and reduced intensity, suggesting partial amorphization and uniform dispersion within the polymeric matrix.

- XRD patterns of pure mesalazine showed sharp crystalline peaks, while the optimized microspheres exhibited reduced or diffused peaks, confirming the transition to a more amorphous state, which favors sustained release.

These analyses validate the compatibility of the drug with the excipients and confirm successful encapsulation within the hydrogel network.

3.6 In vitro Drug Release Profile

Drug release studies conducted at pH 1.2 (2 hours), pH 6.8 (3 hours), and pH 7.4 (up to 12 hours) showed minimal release in acidic conditions and sustained release in colonic pH. This behavior aligns with the swelling profile and supports colon-targeted delivery.

Table 8: In vitro Drug Release of Optimized Microspheres

Time (h)	pH 1.2	pH 6.8	pH 7.4
0	0	0	0
2	9.4 ± 0.6	-	-
5	-	35.2 ± 1.8	-
12	-	-	89.5 ± 2.1

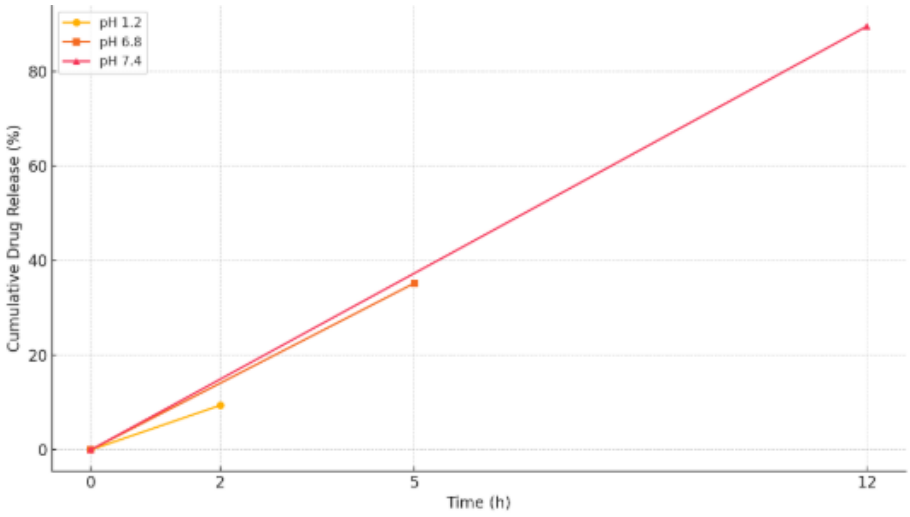

Figure 4: Cumulative Drug Release Profile in Different pH Media

(*Insert a line graph showing cumulative % release vs. time for pH 1.2, 6.8, and 7.4*)

Kinetic Modeling

The release data were fitted to different kinetic models. The Korsmeyer–Peppas model provided the best fit ($R^2 > 0.98$), indicating a non-Fickian (anomalous) transport mechanism, which combines diffusion and polymer relaxation.

Table 9: Drug Release Kinetics of Optimized Microspheres

Model	**R^2 Value**
Zero Order	0.921
First Order	0.936
Higuchi	0.951
Korsmeyer–Peppas	0.987

4. Conclusion

The present study successfully demonstrated the design, optimization, and characterization of pH-responsive hydrogel microspheres for the colon-specific delivery of mesalazine. Using a systematic experimental design approach, the formulation was optimized to achieve high encapsulation efficiency (78.2%), controlled particle size (~5.1 µm), and targeted drug release behavior. Swelling and in vitro release studies confirmed the pH-responsive nature of the microspheres, with minimal release in acidic conditions and sustained release at colonic pH, aligning well with the desired drug delivery profile for inflammatory bowel conditions such as ulcerative colitis and Crohn's disease (Tiwari et al., 2021).

Advanced characterization techniques including FTIR, DSC/TGA, and XRD confirmed the successful encapsulation of mesalazine and demonstrated strong

compatibility between the drug and polymer matrix. The Korsmeyer–Peppas kinetic model best described the drug release mechanism, indicating an anomalous transport system driven by both diffusion and matrix erosion (Makhlof et al., 2015). Additionally, the microspheres exhibited good stability under accelerated storage conditions, further supporting their suitability for pharmaceutical application.

The implications of this research are promising, suggesting that optimized pH-responsive hydrogel microspheres can serve as an effective oral colon-targeted drug delivery system. This system could significantly reduce systemic side effects and improve therapeutic outcomes for patients with colonic diseases. However, further in vivo studies and clinical trials are required to establish pharmacokinetic parameters, therapeutic efficacy, and long-term safety (Sinha & Kumria, 2001).

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