

PREPARATION AND CHARACTERIZATION OF POLYMER BASED MICROSPHERE FORMULATION CONTAINING COMBINE DRUGS FOR GASTRO PROTECTIVE ACTIVITY

Umit Kumar Pandey, Priyanka Rathore, Ruchi Kapoor, Surbhi Jain,

RB Goswami, Neelima Goswami*

Sagar Institute of Research Technology & Science – Pharmacy, Ayodhya Bypass Road, Bhopal (M.P.)

Corresponding author mail id: maanyana@gmail.com

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Abstract

The present study aimed to develop and characterize polymer-based microspheres containing a combination of Lafutidine and Aloin for enhanced gastroprotective activity and controlled drug delivery. Microspheres were prepared using the emulsification solvent evaporation method employing chitosan and sodium alginate as biodegradable polymers. The prepared formulations were evaluated for physical appearance, particle size, zeta potential, drug entrapment efficiency, surface morphology, in vitro drug release, and stability. UV spectrophotometric analysis showed $\lambda_{\max}$ values of 274.0 nm for Lafutidine and 252.5 nm for Aloin. The prepared microspheres were spherical, free-flowing, and exhibited particle sizes ranging from 241.4 ± 1.8 to 804.4 ± 4.2 nm. Among all formulations, MSF2 demonstrated the most desirable characteristics, with a particle size of 241.4 ± 1.8 nm, zeta potential of $+54.9 \pm 0.5$ mV, and entrapment efficiency of $93.75 \pm 0.65\%$. SEM analysis confirmed the formation of spherical microspheres with smooth surfaces. The optimized formulation exhibited sustained drug release, achieving 94.49% cumulative release over 12 hours. Drug release kinetics followed the Zero-Order model ($R^2 = 0.973$), indicating a controlled release pattern. Stability studies conducted under long-term and accelerated conditions demonstrated minimal changes in particle size and drug entrapment efficiency over 90 days. The results suggest that Lafutidine and Aloin-loaded polymeric microspheres represent a promising gastroprotective drug delivery system capable of improving therapeutic efficacy, prolonging drug release, and enhancing patient compliance.

Introduction

Gastric ulcer is a common gastrointestinal disorder characterized by erosion of the gastric mucosal lining due to an imbalance between aggressive factors such as gastric acid, pepsin, reactive oxygen species, *Helicobacter pylori* infection, and nonsteroidal anti-inflammatory drugs (NSAIDs), and protective factors including mucus, bicarbonate secretion, prostaglandins, mucosal blood flow, and cellular regeneration [1-4].

Despite the availability of several anti-ulcer medications, gastric ulcer disease continues to be a significant global health problem because of its high prevalence, recurrence rate, and associated complications such as bleeding, perforation, and gastric carcinoma [5-9].

Current pharmacological management of gastric ulcers includes proton pump inhibitors, H_2 -receptor antagonists, antacids, mucosal protective agents, and antibiotics

for *H. pylori* eradication [10-11]. Although these therapies are effective, prolonged use may lead to adverse effects such as vitamin B12 deficiency, hypomagnesemia, drug interactions, antibiotic resistance, and relapse after discontinuation. Therefore, there is a growing interest in developing novel drug delivery systems and combination therapies that can improve therapeutic efficacy while minimizing adverse effects [12].

Lafutidine is a second-generation histamine H₂-receptor antagonist widely used in the treatment of gastric and duodenal ulcers. In addition to suppressing gastric acid secretion, lafutidine exhibits unique gastroprotective properties by stimulating capsaicin-sensitive sensory neurons, enhancing gastric mucosal blood flow, increasing mucus secretion, and promoting mucosal defense mechanisms. These additional protective effects distinguish lafutidine from conventional H₂-receptor antagonists and make it a promising candidate for ulcer management [13].

Aloin is a naturally occurring anthraquinone glycoside primarily isolated from species of *Aloe*. Besides its well-known medicinal properties, aloin possesses antioxidant, anti-inflammatory, wound-healing, and cytoprotective activities. Several studies

have demonstrated that aloin can reduce oxidative stress, inhibit inflammatory mediators, and protect gastric mucosal tissues against chemically induced damage. The antioxidant potential of aloin may contribute significantly to the prevention of gastric ulceration by scavenging reactive oxygen species and preserving the integrity of the gastric mucosa [14].

The combination of lafutidine and aloin is expected to provide a synergistic gastroprotective effect through multiple mechanisms, including acid suppression, enhancement of mucosal defense, reduction of oxidative stress, and inhibition of inflammatory pathways. However, achieving effective and sustained delivery of these therapeutic agents remains a challenge due to variations in gastric residence time and drug release characteristics [15].

Microsphere-based drug delivery systems have emerged as promising carriers for controlled and targeted drug delivery. Polymeric microspheres offer several advantages, including improved drug stability, controlled and sustained drug release, enhanced bioavailability, reduced dosing frequency, and prolonged gastric retention. These systems can maintain therapeutic drug concentrations at the site of action for extended periods, thereby

improving treatment outcomes and patient compliance. Various biodegradable and biocompatible polymers such as ethyl cellulose, Eudragit, sodium alginate, chitosan, and hydroxypropyl methylcellulose have been extensively employed in microsphere formulations because of their excellent drug encapsulation and release-modulating properties [16].

In the present study, polymer-based microspheres containing lafutidine and aloin were developed with the objective of achieving controlled drug release and enhanced gastroprotective activity. The formulated microspheres were characterized for particle size, surface morphology, drug entrapment efficiency, production yield, flow properties, in vitro drug release behavior, and stability characteristics. Furthermore, the gastroprotective potential of the optimized formulation was evaluated to determine its effectiveness in protecting the gastric mucosa. The development of such a dual-drug microsphere system may provide a novel and effective therapeutic approach for the management of gastric ulcer disease.

Material and Methods

Material

Lafutidine and Aloin were used as the active pharmaceutical ingredients for the preparation of gastroprotective microspheres. Chitosan and sodium alginate served as biodegradable polymers for microsphere formation. Polyvinyl alcohol (PVA) was employed as a stabilizing agent in the aqueous phase, while chloroform was used as the organic solvent during the emulsification process. Methanol was utilized for drug analysis and calibration studies. All chemicals and reagents used in the study were of analytical grade and were used without further purification.

Methods

Determination of $\lambda_{\max}$ of Lafutidine and Aloin

The maximum absorption wavelength ($\lambda_{\max}$) of Lafutidine and Aloin was determined using a UV–Visible spectrophotometer. Accurately weighed quantities of each drug were dissolved separately in methanol to prepare standard stock solutions. Appropriate dilutions were then prepared and scanned over the wavelength range of 200–400 nm using methanol as the blank. The wavelength corresponding to the maximum absorbance was recorded as the $\lambda_{\max}$ and subsequently utilized for quantitative estimation of the drugs in all analytical studies [17].

Preparation of Calibration Curves

Calibration curves of Lafutidine and Aloin were constructed to establish the relationship between concentration and absorbance. Standard stock solutions (100 μ g/mL) of both drugs were prepared in methanol ^[18]. Serial dilutions were made to obtain concentrations ranging from 2–12 μ g/mL for Lafutidine and 20–100 μ g/mL for Aloin. The absorbance of each solution was measured at the respective λ_{max} values using a UV–Visible spectrophotometer. Calibration plots were generated by plotting absorbance against concentration, and the regression equation along with the correlation coefficient (R^2) was calculated. The obtained calibration curves were used for drug content determination and in vitro release studies.

Preparation of Lafutidine and Aloin-Loaded Microspheres

Lafutidine and Aloin-loaded microspheres were prepared by the emulsification solvent evaporation technique. Chitosan and sodium alginate were selected as polymeric carriers,

while polyvinyl alcohol (PVA) served as the stabilizing agent. Equal quantities of Lafutidine and Aloin (10 mg each) were incorporated into the polymeric solution ^[19]. Chitosan was dissolved in 1% acetic acid, whereas sodium alginate was dissolved separately to obtain a homogeneous polymeric dispersion. The drug mixture was dispersed uniformly within the polymer solution. The aqueous phase was prepared by dissolving 0.3% w/v PVA in distilled water under continuous stirring.

The drug-polymer dispersion was added dropwise into the aqueous phase under mechanical stirring at 500 rpm. Stirring was continued for 3 h at room temperature ($25 \pm 2^\circ\text{C}$) to facilitate solvent evaporation and microsphere formation. The resulting microspheres were collected by filtration, washed repeatedly with deionized water to remove residual PVA and untrapped drug, and dried at room temperature for 24 h. The dried microspheres were stored in a desiccator until further evaluation ^[20-23].

Table 1: Composition of Lafutidine and Aloin-Loaded Microspheres

Formulation	Drug Ratio (Lafutidine: Aloin)	Chitosan (mg)	Sodium Alginate (mg)	Chloroform (mL)	PVA (% w/v)	Aqueous Phase (mL)
F1	1:1	50	300	20	0.3	15
F2	1:1	100	250	20	0.3	15

F3	1:1	150	200	20	0.3	15
F4	1:1	200	150	20	0.3	15
F5	1:1	250	100	20	0.3	15

Evaluation of Lafutidine and Aloin-Loaded Microspheres

Physical Appearance

The prepared microspheres were visually examined for color, shape, surface characteristics, texture, and aggregation. A small quantity of microspheres was placed on a glass slide and observed under normal daylight. Preliminary assessment of particle morphology was performed using optical microscopy, while detailed surface characterization was carried out using Scanning Electron Microscopy (SEM). The observations provided information regarding the physical appearance and uniformity of the prepared microspheres ^[24].

Particle Size Analysis

Particle size and polydispersity index (PDI) were determined using Dynamic Light Scattering (DLS). Microspheres were dispersed in distilled water and sonicated briefly to obtain a uniform suspension. The dispersion was transferred into a clean cuvette and analyzed at room temperature. The average hydrodynamic diameter and PDI were recorded to evaluate particle size distribution and formulation uniformity ^[25].

Zeta Potential Measurement

The surface charge and colloidal stability of the microspheres were evaluated by zeta potential analysis using a zeta potential analyzer based on electrophoretic light scattering. Microspheres were dispersed in deionized water and transferred into a zeta cell. Measurements were performed at 25°C, and the average zeta potential values were recorded in millivolts (mV). Higher absolute zeta potential values were considered indicative of improved physical stability and reduced particle aggregation ^[26-27].

Drug Entrapment Efficiency

Drug entrapment efficiency (%EE) was determined by extracting the entrapped drugs from the microspheres using methanol. An accurately weighed quantity of microspheres was dispersed in methanol and subjected to stirring and sonication to ensure complete drug extraction. The mixture was filtered, and the filtrate was analyzed spectrophotometrically at the respective λ_{max} values. The percentage entrapment efficiency was calculated using the following equation:

$$\%EE = \frac{\text{Actual Drug Content}}{\text{Theoretical Drug Content}} \times 100$$

The obtained values indicated the efficiency of the polymeric matrix in encapsulating Lafutidine and Aloin within the microspheres [28-29].

Scanning Electron Microscopy (SEM)

The morphology of the microspheres was evaluated using SEM. A small amount of microspheres from the optimized formulation was mounted onto aluminum stubs using double-sided adhesive tape and coated with a thin layer of gold under vacuum. The samples were examined at suitable magnifications to assess particle shape, surface texture, porosity, and structural integrity. SEM analysis provided detailed information regarding the surface morphology and spherical nature of the microspheres [30-31].

In Vitro Drug Release Study

The release behavior of Lafutidine and Aloin from the microspheres was investigated using the dialysis bag diffusion method [32]. An accurately weighed quantity of microspheres was placed inside a pre-soaked dialysis membrane and immersed in 100 mL phosphate buffer solution maintained at $37 \pm 2^\circ\text{C}$ under continuous stirring at 100 rpm. At predetermined time intervals (0, 1, 3, 5, 7, 9, 11, 13, and 15 h), 2

mL samples were withdrawn and replaced with an equal volume of fresh dissolution medium to maintain sink conditions. The collected samples were analyzed spectrophotometrically at the respective λ_{max} values, and cumulative percentage drug release was calculated and plotted against time [33-34].

Stability Studies

The optimized microsphere formulation was subjected to stability studies according to ICH Q1A(R2) guidelines. Samples were packed in airtight containers and stored under accelerated conditions ($40 \pm 2^\circ\text{C}/75 \pm 5\% \text{ RH}$) as well as room temperature conditions. The formulations were evaluated periodically for physical appearance, drug content, entrapment efficiency, particle size, and in vitro drug release behavior. The stability data were used to assess the long-term storage suitability and overall stability of the microsphere formulation [35-36].

Results and Discussion

The present study successfully developed and characterized polymer-based microspheres containing a combination of Lafutidine and Aloin for gastroprotective activity using the emulsification solvent evaporation technique. The prepared

microspheres exhibited satisfactory physicochemical characteristics, high drug entrapment efficiency, controlled drug release behavior, and good stability, indicating their suitability as an oral controlled-release gastroprotective drug delivery system.

The UV spectrophotometric analysis revealed that the $\lambda_{\max}$ of Lafutidine and Aloin were observed at 274.0 nm and 252.5 nm, respectively, while the combined drug formulation exhibited maximum absorbance at 282.0 nm (Figure 1–3). These wavelengths were subsequently used for quantitative drug estimation throughout the study.

The physical appearance evaluation demonstrated that all prepared microsphere formulations were off-white to pale yellow in color, odorless, spherical, free-flowing, and solid in nature (Table 2). Such characteristics indicate successful microsphere formation with acceptable aesthetic and handling properties.

Particle size analysis showed that the microspheres possessed sizes ranging from 241.4 ± 1.8 nm to 804.4 ± 4.2 nm (Table 3). Among all formulations, MSF2 exhibited the smallest particle size (241.4 ± 1.8 nm), which may be attributed to the optimum polymer ratio and efficient emulsification

process. Smaller particle size generally enhances surface area, improves dissolution characteristics, and contributes to uniform drug release.

Zeta potential measurements revealed positive surface charges ranging from $+25.6 \pm 0.3$ mV to $+54.9 \pm 0.5$ mV (Table 4). The optimized formulation MSF2 showed the highest zeta potential value ($+54.9 \pm 0.5$ mV), indicating excellent colloidal stability due to strong electrostatic repulsion between particles. A zeta potential value greater than ± 30 mV is generally considered indicative of a stable particulate system. The zeta potential distribution of the optimized formulation is presented in Figure 4.

Scanning Electron Microscopy (SEM) analysis further confirmed the successful formation of microspheres with a nearly spherical shape and smooth surface morphology (Figure 5). The absence of visible cracks or surface irregularities suggested uniform drug encapsulation within the polymeric matrix and structural integrity of the microspheres.

Drug entrapment efficiency is an important parameter for evaluating the encapsulation capability of the polymeric system. The entrapment efficiency values ranged from $69.43 \pm 0.59\%$ to $93.75 \pm 0.65\%$ (Table 5). Among all formulations, MSF2 exhibited

the highest entrapment efficiency ($93.75 \pm 0.65\%$), indicating superior drug retention within the polymer matrix. The higher entrapment efficiency may be attributed to the optimized ratio of chitosan and sodium alginate, which provided sufficient matrix density to encapsulate both Lafutidine and Aloin effectively.

The in vitro drug release study of the optimized formulation (MSF2) demonstrated a sustained and controlled release pattern over 12 hours (Table 6). The cumulative drug release gradually increased from 22.36% at 2 hours to 94.49% at 12 hours, indicating prolonged drug availability. Such controlled release behavior is beneficial for gastroprotective therapy as it may reduce dosing frequency and maintain therapeutic drug concentrations for an extended period.

Drug release kinetic analysis revealed that the release data best fitted the Zero-Order kinetic model with the highest correlation coefficient ($R^2 = 0.973$), followed by First-Order ($R^2 = 0.887$), Higuchi ($R^2 = 0.868$), and Korsmeyer–Peppas ($R^2 = 0.798$) models (Table 7). These findings suggest that the optimized microsphere formulation predominantly follows zero-order release kinetics, providing a constant drug release rate independent of drug concentration. Such

behavior is highly desirable for controlled-release pharmaceutical formulations.

The stability study conducted under both long-term ($25^\circ\text{C} \pm 2^\circ\text{C}/60 \pm 5\% \text{ RH}$) and accelerated ($40^\circ\text{C} \pm 2^\circ\text{C}/70 \pm 5\% \text{ RH}$) conditions demonstrated minimal changes in particle size and entrapment efficiency over a period of 90 days (Table 8). The particle size increased only slightly from 241.4 nm to 248.0 nm under long-term conditions and to 251.0 nm under accelerated conditions. Similarly, entrapment efficiency remained above 91% throughout the study period. These results indicate that the optimized formulation possesses excellent physical and chemical stability and is suitable for long-term storage.

The findings suggest that formulation MSF2 exhibited the most favorable characteristics among all prepared formulations, including minimum particle size, maximum zeta potential, highest entrapment efficiency, sustained drug release, and excellent stability. The combination of Lafutidine and Aloin encapsulated within polymeric microspheres may therefore provide an effective gastroprotective drug delivery system with enhanced therapeutic efficacy and patient compliance.

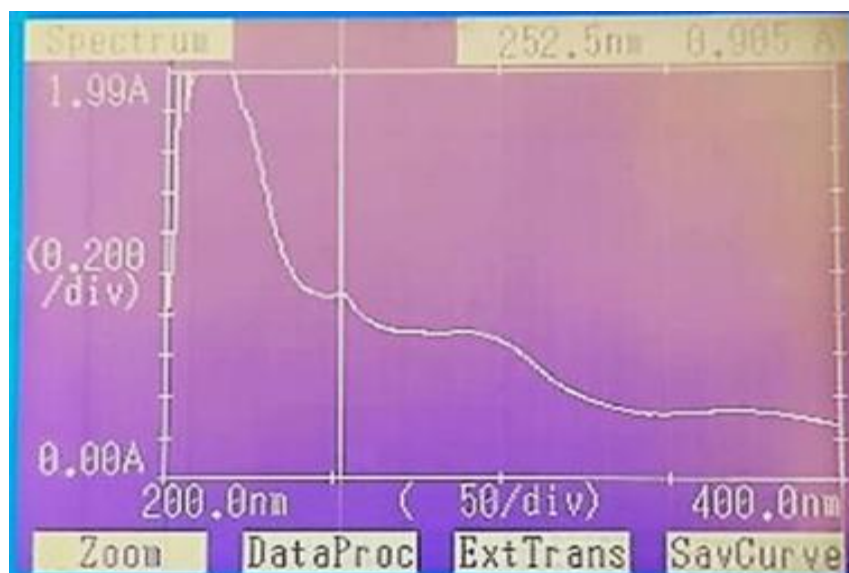


Figure 1: $\lambda_{\max}$ of Lafutidine (274.0 nm)

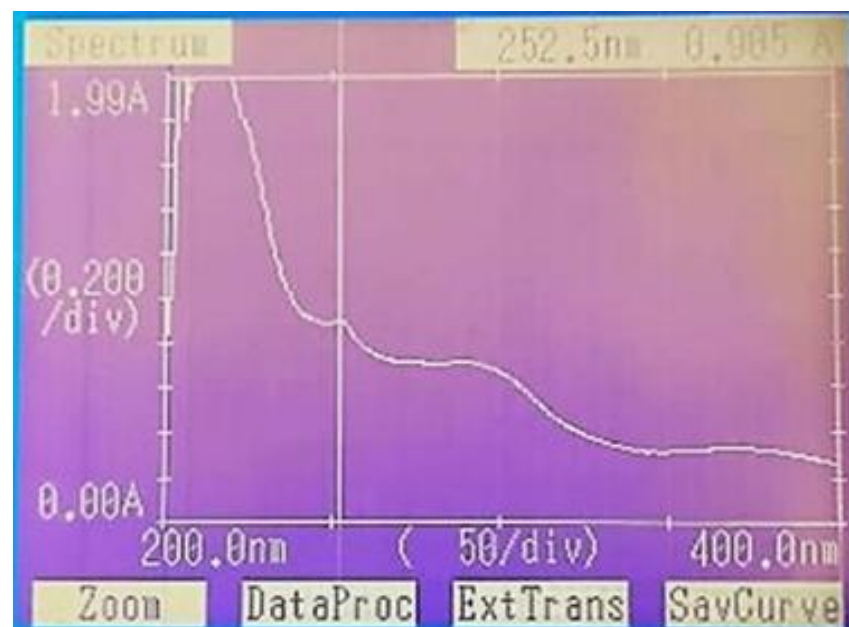


Figure 3: $\lambda_{\max}$ of Aloin (252.5 nm)

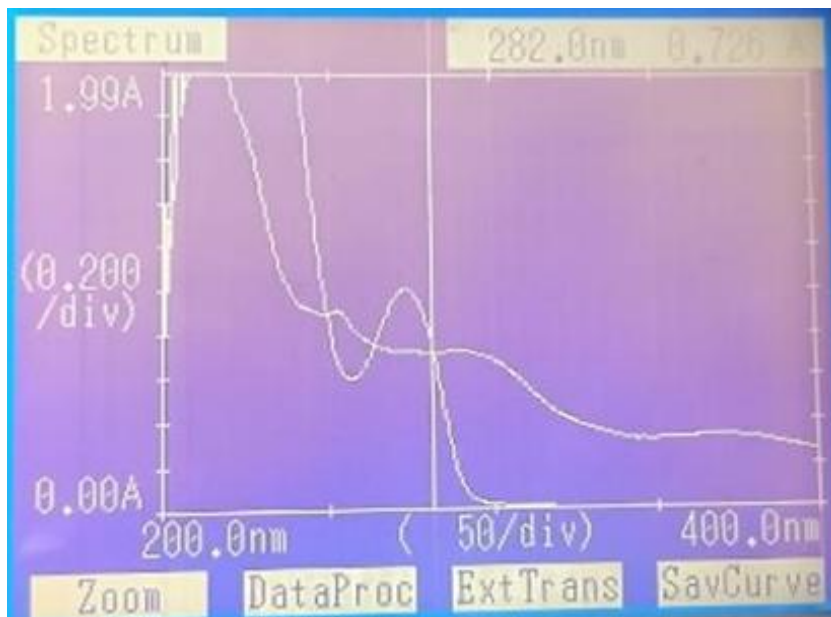


Figure 3: λ_{max} of Lafutidine and Aloin (282.0 nm)

Table 2: Physical Appearance of Lafutidine and Aloin-Loaded Microspheres

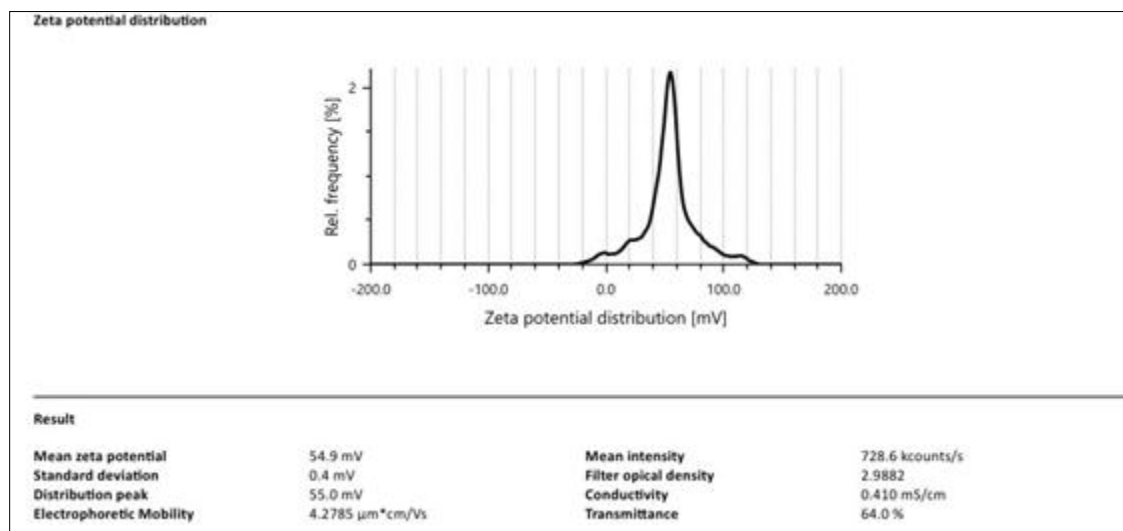
S. No.	Parameter	Observation
1	Color	Off-white to pale yellow
2	Odor	Odorless
3	Appearance	Spherical and free-flowing
4	Physical State	Solid

Table 3: Particle Size of Lafutidine and Aloin-Loaded Microspheres

S. No.	Formulation Code	Particle Size (nm)
1	MSF1	286.7 ± 2.4
2	MSF2	241.4 ± 1.8
3	MSF3	477.3 ± 3.1
4	MSF4	308.9 ± 2.6
5	MSF5	804.4 ± 4.2

Table 4: Zeta Potential of Lafutidine and Aloin-Loaded Microspheres

S. No.	Formulation Code	Zeta Potential (mV)
1	MSF1	+36.3 ± 0.4
2	MSF2	+54.9 ± 0.5
3	MSF3	+25.6 ± 0.3
4	MSF4	+47.2 ± 0.4
5	MSF5	+50.5 ± 0.6

**Figure 4: Zeta Potential MSF2**

**Figure 5: Scanning electron microscope (SEM)****Table 5: Entrapment Efficiency of Lafutidine and Aloin-Loaded Microspheres**

S. No.	Formulation Code	Entrapment Efficiency (%)
1	MSF1	75.40 ± 0.72
2	MSF2	93.75 ± 0.65
3	MSF3	80.38 ± 0.54
4	MSF4	86.62 ± 0.48
5	MSF5	69.43 ± 0.59

Table 6: In Vitro Drug Release Profile of Optimized Microsphere Formulation (MSF2)

Time (h)	Cumulative Drug Release (%)	Drug Remaining (%)	√Time	Log Drug Remaining	Log Time	Log Cumulative Drug Release
0	0.00	100.00	0.000	2.000	0.000	0.000
2	22.36	77.64	1.414	1.890	0.301	1.349
3	32.40	67.60	1.732	1.830	0.477	1.511
4	43.38	56.62	2.000	1.753	0.602	1.637
5	54.42	45.58	2.236	1.659	0.699	1.736
7	64.46	35.54	2.646	1.551	0.845	1.809

9	74.52	25.48	3.000	1.406	0.954	1.872
10	83.54	16.46	3.162	1.216	1.000	1.922
12	94.49	5.51	3.464	0.741	1.079	1.975

Table 7: Drug Release Kinetics of Optimized Microsphere Formulation (MSF2)

Formulation	Release Kinetic Model	Correlation Coefficient (R ²)
MSF2	Zero-Order	0.973
MSF2	First-Order	0.887
MSF2	Higuchi Model	0.868
MSF2	Korsmeyer–Peppas Model	0.798

Table 8: Stability Study of Optimized Microsphere Formulation (MSF2)

Time (Days)	25°C ±2°C & 60 ±5% RH		40°C ±2°C & 70 ±5% RH	
	Particle Size (nm)	Entrapment Efficiency (%)	Particle Size (nm)	Entrapment Efficiency (%)
0	241.4	93.75	241.4	93.75
30	243.1	91.50	244.0	96.40
45	244.8	94.20	246.2	91.00
60	246.5	93.90	248.5	92.70
90	248.0	95.60	251.0	95.30

Conclusion

The present study successfully developed Lafutidine and Aloin-loaded polymeric microspheres using chitosan and sodium alginate through the emulsification solvent evaporation method. Among all formulations, MSF2 exhibited the most favorable characteristics, including optimum particle size, high zeta potential, maximum

entrapment efficiency, and sustained drug release. The optimized formulation showed controlled drug release for up to 12 hours and remained stable under both long-term and accelerated storage conditions. These findings indicate that the developed microsphere system has significant potential as an effective gastroprotective drug delivery platform, offering prolonged

therapeutic action, improved drug stability, and enhanced patient compliance.

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