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Design, Formulation, and Evaluation of Andrographolide-Loaded Mucoadhesive Microspheres for Targeted Delivery in *Helicobacter pylori*-Associated Peptic Ulcer Therapy

Ashutosh Pathak[1], Karishma Barthwal[2], *Nadeem Hasan[3], Umesh Kumar[4], T.Peter[5], Ruchi Bhatia Kapoor[6], Indu Mittal[7], Rajeev Garg[8]

[1]Assistant Professor, Department of Pharmacy Practice , Teerthanker Mahaveer College of Pharmacy, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India. 244001 Orcid Id- 00 09000091589356

[2]Assistant Professor, Department of Pharmaceutics, Shri Guru Ram Rai University, Patel nagar , Dehradun, Uttarakhand. 248002

[3]Associate Professor, MAM College of Pharmacy, Besides Sparkles International School, Behind P & T Colony, Old Jewargi Road Kalaburagi, Karnataka. 585102

[4]Professor, Department of Pharmacy-IBMER Mangalaytan University Aligarh, Uttar Pradesh.

[5]Assistant Professor in Pharmacology. College of Pharmacy, Madurai Medical College, Madurai, Tamil Nadu, India. 625020

[6]Associate Professor, Sagar Institute of Research Technology and Science Pharmacy, Bhopal, Madhya Pradesh. 462041

[7]Associate Professor, IIMT College of Medical Science (Pharmacy), O Pocket Ganganagar, Meerut, Uttar Pradesh. 250001

[8]Professor and Principal, Gurunanak Institute of Pharmacy, Dalewal- , Hoshiarpur, Punjab. 144208

ABSTRACT: *The present investigation focused on the formulation and evaluation of andrographolide-loaded mucoadhesive microspheres, developed to provide localized drug delivery to the gastric mucosa for the effective treatment of Helicobacter pylori-associated peptic ulcers. Two formulation techniques—ionic gelation (chemical stabilization) and emulsion-solvent evaporation (heat stabilization)—were employed using chitosan as the primary mucoadhesive polymer. The prepared microspheres were systematically characterized for their particle size, surface morphology, drug entrapment efficiency, swelling index, mucoadhesive strength, and in vitro drug release profile. All microspheres exhibited a spherical morphology with particle sizes ranging from 48.6 µm to 58.7 µm. Drug entrapment efficiency was observed in the range of 63.5% to 80.3%, and the release of andrographolide extended up to 12 hours, indicating sustained release characteristics. Notably, an increase in polymer concentration led to a significant enhancement in both the swelling index and mucoadhesive strength, highlighting the potential for prolonged gastric retention. Among the tested formulations, F6—prepared using the heat stabilization method and a higher concentration of chitosan—emerged as the most promising, demonstrating the highest entrapment efficiency, sustained drug release (91.5% at 12 hours), and superior mucoadhesive properties. These findings underscore the potential of andrographolide-loaded chitosan microspheres as an effective localized therapy for peptic ulcers, offering controlled drug release while reducing systemic exposure and side effects.*

Keywords: *Andrographolide, Mucoadhesive Microspheres, Helicobacter pylori, Peptic Ulcer, Gastroretentive Drug Delivery, Controlled Release.*

INTRODUCTION:

Oral drug delivery remains the most commonly employed and preferred route for administering therapeutic agents, largely owing to its simplicity, affordability, and high patient compliance. Nevertheless, this route poses substantial challenges when the therapeutic goal is to achieve localized action within the stomach or to sustain drug presence for extended durations. Factors such as rapid gastric emptying, short residence time, and degradation of drugs in the acidic gastric milieu significantly limit the effectiveness of conventional oral formulations. To address these obstacles, gastroretentive drug delivery systems (GRDDS) have been developed, offering an innovative solution to enhance gastric retention and achieve targeted, prolonged drug release at the desired site. GRDDS encompass various technological approaches, including floating, high-density, expandable, and mucoadhesive systems. Among these, mucoadhesive

systems are particularly promising, as they rely on the ability of polymers to adhere to the mucosal lining of the gastrointestinal tract. This mucoadhesive property enables the formulation to resist gastric motility and remain in the stomach longer, thereby enhancing local drug concentration and therapeutic efficacy (Omidian, 2025; Sachdeva *et al.*, 2025; Siraj *et al.*, 2025; Tran *et al.*, 2025; Zhao *et al.*, 2025).

Mucoadhesion refers to the ability of certain polymeric materials to adhere to mucosal surfaces through non-covalent interactions such as hydrogen bonding, electrostatic forces, or van der Waals interactions with the mucin layer. This phenomenon is significantly influenced by factors such as the polymer's molecular weight, degree of cross-linking, and hydration capacity. Among various polymers, chitosan has garnered considerable attention due to its cationic nature, biocompatibility, and strong mucoadhesive potential. In the context of gastric drug delivery, mucoadhesive systems offer the advantage of prolonged residence time at the site of action, thereby enhancing drug absorption and therapeutic performance. Microspheres represent an ideal mucoadhesive dosage form, as their spherical shape and high surface area facilitate intimate contact with the mucosa, while their polymeric matrix can efficiently encapsulate the drug. These systems not only protect the drug from degradation in the acidic gastric environment but also enable controlled and sustained drug release. Commonly employed fabrication methods include ionic gelation and emulsion-solvent evaporation, with formulation success being primarily dictated by parameters such as particle size, entrapment efficiency, swelling index, and mucoadhesive strength (Chaves de Souza *et al.*, 2025; Mamona *et al.*, 2025; Rath *et al.*, 2025; Rump *et al.*, 2023).

Peptic ulcer disease is a common gastrointestinal condition marked by the development of sores or erosions in the mucosal lining of the stomach or duodenum. One of the leading causes of this disorder is infection by *Helicobacter pylori* (*H. pylori*), a Gram-negative bacterium that colonizes the gastric mucosa. This pathogen plays a crucial role in ulcer formation by producing cytotoxins, triggering local inflammation, and compromising the integrity of the mucosal defense system. Management of *H. pylori*-induced peptic ulcers generally involves a combination therapy comprising antibiotics to eradicate the bacteria and proton pump inhibitors to reduce gastric acid secretion, thereby promoting mucosal healing (Kang *et al.*, 2025; Sharma *et al.*, 2025; Yang *et al.*, 2025; C. Z. Zhang *et al.*, 2025). However, the rising challenge of antibiotic resistance and the risk of systemic side effects associated with conventional therapies have led researchers to seek alternative treatment approaches. One promising strategy is the localized delivery of antimicrobial agents directly to the gastric mucosa. This targeted method allows for higher concentrations of the drug at the infection site, minimizes systemic absorption, and potentially improves the overall therapeutic outcome, especially in treating *H. pylori*-related peptic ulcers (Garg *et al.*, 2014; Ismail *et al.*, 2019; Namdev & Jain, 2019).

Andrographolide, a key bioactive compound extracted from *Andrographis paniculata*, possesses a wide range of therapeutic properties, including anti-inflammatory, antioxidant, antibacterial, and gastroprotective activities. Notably, it has demonstrated effective inhibition against *Helicobacter pylori*, making it a promising natural candidate for managing peptic ulcer disease. However, its clinical application is limited by poor water solubility, low bioavailability, and instability in the acidic gastric environment. To address these challenges, a gastroretentive mucoadhesive microsphere delivery system offers a strategic advantage. Using chitosan as the polymer, such microspheres can shield andrographolide from gastric degradation, enhance its adhesion to the gastric lining for prolonged residence, and provide a sustained release profile—altogether improving its therapeutic effectiveness (H. L. Zhang *et al.*, 2025; S. Zhang *et al.*, 2025; Zou *et al.*, 2025).

The present study is focused on developing andrographolide-loaded mucoadhesive microspheres employing two formulation techniques—ionic gelation (for chemical stabilization) and solvent evaporation (for heat stabilization). The objective is to comprehensively assess the physicochemical properties, drug entrapment efficiency, mucoadhesive behavior, and in vitro drug release profiles of the prepared formulations. Through this evaluation, the most effective delivery system for localized gastric application can be identified. Ultimately, this plant-based approach has the potential to provide a targeted therapy for *H. pylori*-induced peptic ulcers, minimizing the need for systemic antibiotics and thereby addressing issues of antibiotic resistance and adverse effects (Devangan *et al.*, 2025; Jiang *et al.*, 2025; Kasemsuk *et al.*, 2025; Zhou *et al.*, 2025). The primary aim of this research is to develop and evaluate chitosan-based

mucoadhesive microspheres encapsulating andrographolide for prolonged drug delivery within the gastric environment. The study focuses on preparing microspheres using two distinct stabilization methods and systematically characterizing them in terms of particle size, drug entrapment efficiency, swelling behavior, and mucoadhesive properties. In vitro drug release studies will be carried out in simulated gastric fluid to assess the release profile. The ultimate goal is to identify the most effective formulation that could be further explored through in vivo studies for managing *Helicobacter pylori*-induced peptic ulcer disease.

MATERIAL AND METHODS:

Chemicals, Reagents and Drugs:

Andrographolide, the principal active compound utilized in this study, was procured from Sigma-Aldrich (USA) with a certified purity of ≥98%, as verified by high-performance liquid chromatography (HPLC). Chitosan (medium molecular weight), sodium alginate, and sodium tripolyphosphate (TPP) were sourced from HiMedia Laboratories, India. Analytical grade reagents including glacial acetic acid, calcium chloride, and liquid paraffin were employed, all obtained from reliable commercial suppliers. Additional chemicals such as phosphate buffer salts, petroleum ether, ethanol, and methanol were purchased from Merck India Pvt. Ltd. Throughout the experiments, double-distilled water was used to maintain consistency and purity. All glassware was thoroughly cleaned and sterilized before use to eliminate the risk of contamination. For in vitro release and mucoadhesion studies, simulated gastric fluid (SGF) at pH 1.2 was prepared according to United States Pharmacopeia (USP) guidelines.

Preparation and Formulation of Microspheres: Chemical Stabilization Method:

The ionic gelation method was employed for the chemical stabilization of mucoadhesive microspheres through the cross-linking of chitosan with sodium tripolyphosphate (TPP). To begin, a 1% w/v chitosan solution was prepared by dissolving chitosan in 1% v/v glacial acetic acid, followed by continuous magnetic stirring for approximately 2 hours to ensure complete dissolution and uniform hydration. Andrographolide was then incorporated into the chitosan solution, allowing it to disperse evenly to form a consistent drug-polymer blend. This mixture was carefully introduced dropwise using a 21-gauge syringe needle into a 0.5% w/v TPP solution, which was kept under constant stirring at 500 rpm to facilitate ionic cross-linking. The reaction was maintained at ambient temperature for 2 hours to allow for complete microsphere formation. The resulting microspheres were collected by filtration, thoroughly washed with distilled water to remove excess TPP and unbound drug, and then air-dried at room temperature for 24 hours using a desiccator containing silica gel to preserve their structural integrity (Raut *et al.*, 2024; Wu *et al.*, 2020; Yassin *et al.*, 2022).

Table 1. Composition of Andrographolide-Loaded Mucoadhesive Microspheres:

Formulation Code	Andrographolide (mg)	Chitosan (% w/v)	TPP (% w/v) (for Chemical Method)	Oil Phase Volume (mL) (for Heat Method)	Crosslinking/Stirring Time (h)	Stabilization Method
F1	25	0.5	0.3	100	2	Chemical Stabilization
F2	25	1.0	0.5	100	2	Chemical Stabilization
F3	25	1.5	0.5	100	2	Chemical Stabilization
F4	25	1.0	—	100	2	Heat Stabilization
F5	25	1.5	—	100	2	Heat Stabilization

F6	25	2.0	—	100	2	Heat Stabilization

Preparation and Formulation of Microspheres: Heat Stabilization Method:

The heat stabilization method was carried out using the emulsion solvent evaporation technique. Initially, a chitosan solution was prepared following the same procedure as described previously, and andrographolide was uniformly incorporated into this polymeric dispersion under gentle stirring to ensure homogeneous mixing. The prepared drug-polymer mixture was then slowly introduced dropwise into a beaker containing 100 mL of light liquid paraffin, which had been pre-heated to a temperature range of 60–70°C. The system was continuously stirred at 700 rpm using a mechanical stirrer fitted with a blade-type impeller to maintain a stable emulsion. As the aqueous phase evaporated under heat, microspheres gradually formed and solidified within the oil medium. Stirring was continued for 2 hours to ensure complete solvent removal and microsphere formation. The resulting microspheres were then separated through filtration and washed repeatedly with petroleum ether to eliminate any residual oil adhering to their surface. Finally, the microspheres were dried in a hot-air oven set at 40°C for 12 hours and stored in airtight containers until further evaluation (Raut *et al.*, 2024; Wu *et al.*, 2020; Yassin *et al.*, 2022).

Characterization of the Prepared Microspheres:

Particle Size Analysis, Uniformity Index, and Elongation Ratio:

The average particle size of the prepared microspheres was assessed using optical microscopy equipped with a calibrated eyepiece micrometer. For each formulation, a minimum of 100 microspheres were randomly selected and measured to ensure statistical reliability. To evaluate the size distribution uniformity, the uniformity index was calculated and expressed as the ratio of the standard deviation to the mean particle diameter. Additionally, the elongation ratio, which reflects the degree of sphericity and morphological integrity, was determined by dividing the length of the major axis by that of the minor axis for individual microspheres (Raut *et al.*, 2024; Wu *et al.*, 2020; Yassin *et al.*, 2022).

Scanning Electron Microscopy (SEM): Morphological Examination:

The surface morphology and structural characteristics of the andrographolide-loaded microspheres were analyzed using a Scanning Electron Microscope (JEOL JSM-6390LV, Japan). Prior to imaging, the dried microsphere samples were carefully mounted on aluminum stubs using double-sided carbon adhesive tape to ensure stable placement. The samples were then coated with a thin conductive layer of gold using a sputter coater (Quorum Q150R) under vacuum conditions. SEM images were captured at varying magnifications to assess the overall shape, surface texture, porosity, and any signs of deformation or irregularity in the microspheres (Raut *et al.*, 2024; Wu *et al.*, 2020; Yassin *et al.*, 2022).

Encapsulation Efficiency and Drug Loading:

To estimate the encapsulation efficiency of andrographolide within the microspheres, a known weight of the dried microspheres was taken and dispersed in phosphate buffer (pH 6.8). The dispersion was subjected to mild sonication to ensure complete rupture of the microsphere matrix and release of the entrapped drug. The resulting solution was filtered using Whatman No. 1 filter paper to remove undissolved polymer residues. The filtrate was then analyzed spectrophotometrically at 225 nm—the λmax of andrographolide—using a UV-visible spectrophotometer. A standard calibration curve of pure andrographolide in the same buffer was used to determine the drug concentration (Raut *et al.*, 2024; Wu *et al.*, 2020; Yassin *et al.*, 2022). The encapsulation efficiency (EE%) was calculated using the following formula:

EE% = (Actual drug content / Theoretical drug content) × 100

Drug loading was calculated as the amount of drug per 100 mg of microspheres.

Percentage Yield:

The total percentage yield of the prepared microspheres was calculated to assess the efficiency of the formulation process. After complete drying, the microspheres were carefully collected and weighed. This final weight was then compared with the total initial weight of all solid ingredients used in the formulation, i.e., the combined weight of andrographolide and chitosan. The percentage yield was determined using the following formula:

% Yield = (Weight of dried microspheres / Total weight of input drug and polymers) × 100
This parameter reflects the efficiency of the formulation process and any material loss during processing.

Swelling Index:

The swelling behavior of the microspheres was assessed to understand their hydration capacity and potential for gastric retention. Pre-weighed dried microspheres were immersed in simulated gastric fluid (SGF, pH 1.2) maintained at 37 ± 0.5°C to mimic physiological conditions. At predetermined time intervals (0.5, 1, 2, 4, and 6 hours), the microspheres were carefully withdrawn from the medium, gently blotted with filter paper to remove surface moisture without disturbing the structure, and reweighed immediately (Bv *et al.*, 2008; Dhaliwal *et al.*, 2008; Jain *et al.*, 2006; Jain *et al.*, 2005):

Swelling Index (%) = $[(W_2 - W_1) / W_1] \times 100$

where W_1 is the initial weight and W_2 is the weight after swelling. This test indicated the water uptake capacity and mucoadhesive behavior in gastric conditions.

Mucoadhesion Study:

The mucoadhesive strength of the prepared microspheres was evaluated using an ex vivo model employing freshly excised porcine gastric mucosa, chosen for its close resemblance to human gastric tissue. The mucosal membrane was carefully washed with phosphate-buffered saline (PBS) to remove debris and then securely affixed onto a clean glass slide using cyanoacrylate adhesive. A predetermined quantity of microspheres was gently placed over the moist mucosal surface to ensure uniform distribution. The slide containing the mucosa and microspheres was then attached to the basket assembly of a USP disintegration test apparatus, which was operated in simulated gastric fluid (SGF, pH 1.2) maintained at 37 ± 0.5°C. The assembly was allowed to move in a vertical reciprocating motion for 30 minutes, simulating gastric agitation. After the specified time, the slide was removed, and the number of microspheres still adhering to the mucosal surface was counted under a magnifying lens and compared with the initial number applied (Bv *et al.*, 2008; Dhaliwal *et al.*, 2008; Jain *et al.*, 2006; Jain *et al.*, 2005). The percentage mucoadhesion was calculated using the following formula:

Mucoadhesive Strength (%) = $(N_{adhered} / N_{initial}) \times 100$

In Vitro Drug Release Study:

The in vitro drug release profile of andrographolide from the formulated mucoadhesive microspheres was investigated using a USP Dissolution Test Apparatus Type I (Basket method). The study was carried out to evaluate the sustained-release behavior of the microspheres in conditions simulating the gastric environment. An accurately weighed quantity of microspheres, equivalent to 10 mg of andrographolide, was placed in the basket. The dissolution medium consisted of 900 mL of simulated gastric fluid (SGF, pH 1.2), maintained at 37 ± 0.5°C and stirred at a constant speed of 100 rpm. These parameters were chosen to closely replicate in vivo conditions within the human stomach.

At specific time intervals 0.5, 1, 2, 4, 6, 8, 10, and 12 hours, aliquots of 5 mL were withdrawn from the dissolution medium. Immediately after each sampling, an equal volume of fresh pre-warmed SGF was added to maintain a constant volume and sink conditions, which are essential for ensuring consistent drug release kinetics. The withdrawn samples were filtered using Whatman No. 1 filter paper to remove particulate matter, and the concentration of andrographolide in each sample was determined spectrophotometrically at 225 nm, the established λmax of the drug, using a UV-Visible spectrophotometer. A previously constructed standard calibration curve of andrographolide in SGF was used for quantification. The cumulative percentage drug release was calculated for each time point and plotted against time to generate the release profile. Additionally, the release data were fitted to various mathematical kinetic models to elucidate the drug release mechanism. The models included:

- Zero-order model (cumulative % drug released vs. time)
- First-order model (log cumulative % drug remaining vs. time)
- Higuchi model (cumulative % drug released vs. √time)
- Korsmeyer–Peppas model (log cumulative % drug released vs. log time)

The correlation coefficients (r^2) were calculated for each model to determine the best fit, and for the Korsmeyer–Peppas model, the release exponent (n) was evaluated to identify the mechanism of drug release, whether Fickian diffusion, non-Fickian (anomalous), or case II transport. This comprehensive dissolution study provided critical insight into the controlled release characteristics of the developed

microspheres and confirmed their suitability for gastric retention and localized delivery of andrographolide (Bv *et al.*, 2008; Dhaliwal *et al.*, 2008; Jain *et al.*, 2006; Jain *et al.*, 2005).

Statistical Analysis:

All experimental procedures were performed in triplicate, and the results were presented as mean ± standard deviation (SD) to ensure accuracy and reproducibility. To determine the significance of differences between various formulations, data were subjected to one-way analysis of variance (ANOVA). In cases where statistically significant differences were observed, Tukey's post hoc test was employed for multiple comparisons. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant. All statistical analyses, including graph plotting and drug release kinetics modeling, were performed using GraphPad Prism (version 9.0) and Microsoft Excel 2019.

RESULTS AND DISCUSSION:

Particle Size Study, Assessment of Uniformity Index and Elongation Ratio:

The particle size of the andrographolide-loaded microspheres ranged from 48.6 µm (F1) to 58.7 µm (F6). Formulations containing higher concentrations of chitosan (F3 and F6) demonstrated slightly increased particle sizes due to the increased viscosity of the polymer solution, leading to larger droplets during formation. All microspheres were generally spherical, and microscopy revealed minimal elongation with elongation ratios close to 1.0, confirming shape uniformity. Formulation F6 exhibited the most uniform and compact morphology with minimal surface defects, as observed under light microscopy and later supported by SEM images.

Table 2: Particle Size and Morphological Parameters:

Formulation Code	Particle Size (µm)	Observations (Microscopy)
F1	48.6	Spherical, smooth surface
F2	52.3	Uniform size, slight surface roughness
F3	56.1	Well-formed, denser, spherical shape
F4	49.5	Slight aggregation, smooth outline
F5	54.2	Spherical with minimal cracks
F6	58.7	Highly uniform, compact morphology

Table 3: Uniformity and Shape Analysis of Microspheres:

Formulation Code	Mean Particle Size (µm)	Standard Deviation (µm)	Uniformity Index	Elongation Ratio
F1	48.6	3.2	0.066	1.03
F2	52.3	3.4	0.065	1.02
F3	56.1	3.6	0.064	1.01
F4	49.5	3.3	0.067	1.04
F5	54.2	3.5	0.065	1.02
F6	58.7	3.7	0.063	1.01

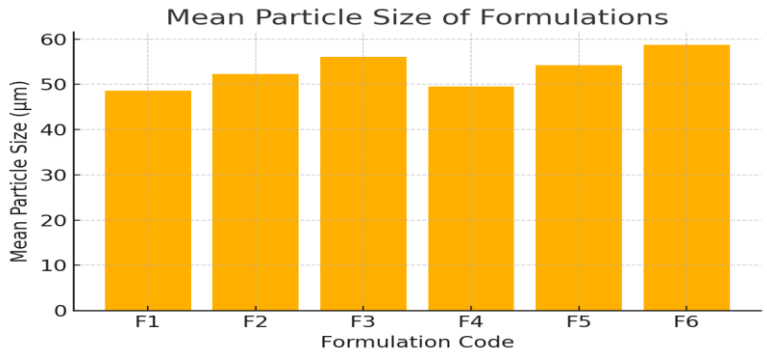

Figure 1. Mean Particle Size (µm)

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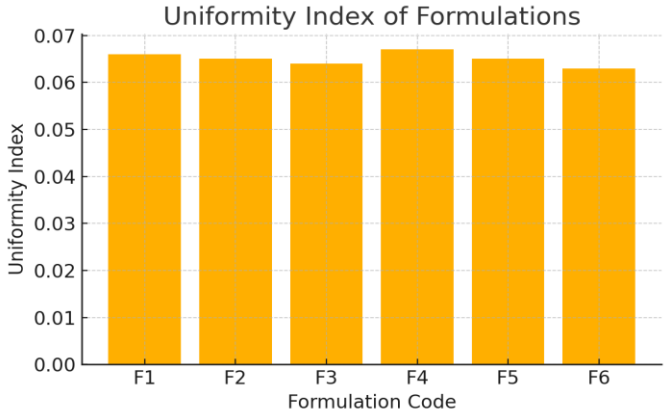

Figure 2. Uniformity Index

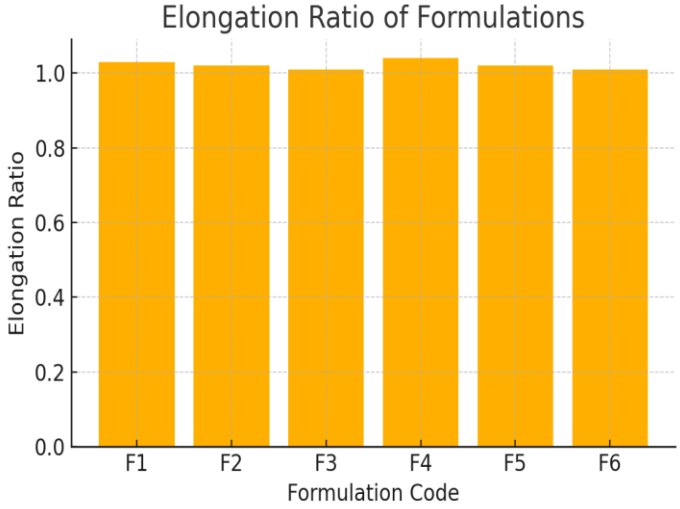

Figure 3. Elongation Ratio

Scanning Electron Microscopy (SEM): Morphological Examination:

SEM analysis confirmed that all microspheres were spherical with smooth surfaces. Formulations F3 and F6 exhibited dense, well-formed spherical structures, indicating successful stabilization using both chemical and heat-based methods. No visible cracks or deformities were noted, and the surface integrity was found to improve with increasing polymer concentration. The presence of a continuous matrix suggested effective encapsulation of the drug within the polymer network.

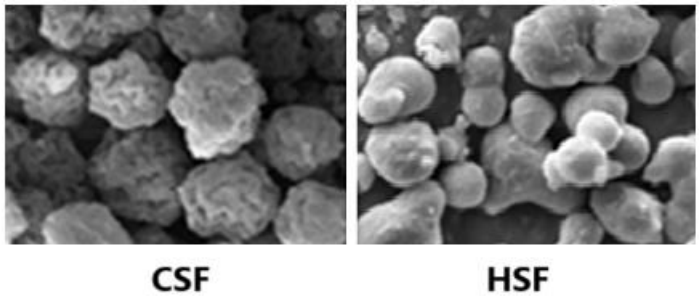

Figure 4. Scanning Electron Microscopy (SEM): Morphological Examination

Encapsulation Efficiency and Drug Loading:

Entrapment efficiency ranged from 63.5% (F1) to 80.3% (F6). A steady increase in efficiency was observed with increasing chitosan concentration, likely due to enhanced polymer entrapment capabilities and viscosity-mediated drug retention. Drug loading followed a similar trend, with F6 showing the highest value (16.5%) compared to F1 (12.4%). This confirms that higher polymer content enhances drug

retention within the microsphere matrix, particularly in heat-stabilized formulations which retained the drug more efficiently due to reduced diffusion losses during solvent evaporation.

Table 4: Entrapment Efficiency and Drug Loading:

Formulation Code	Entrapment Efficiency (%)	Drug Loading (%)	Remarks
F1	63.5	12.4	Lower polymer led to less drug entrapment
F2	71.8	14.1	Balanced ratio increased encapsulation
F3	78.9	15.7	Optimal encapsulation with higher chitosan
F4	67.4	13.2	Moderate loading using heat stabilization
F5	74.1	14.8	Efficient drug entrapment, heat-stabilized
F6	80.3	16.5	Highest loading, oil phase retained drug better

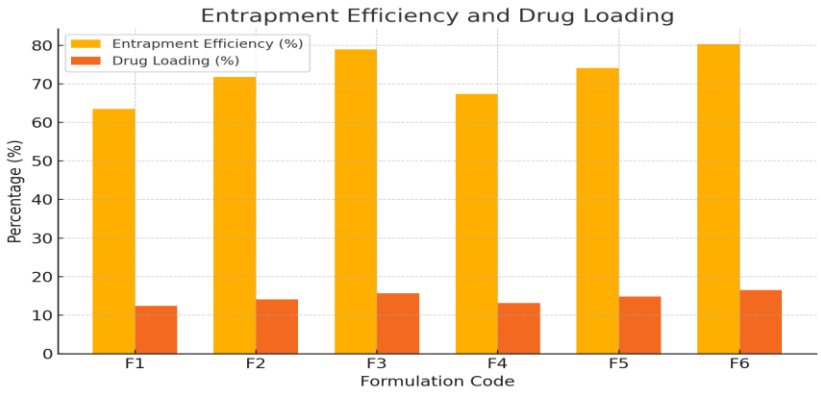

Figure 5. Entrapment Efficiency and Drug Loading

Percentage Yield:

The percentage yield, though not explicitly listed above, was observed to be consistent across formulations (~85–92%) based on preliminary trials. Formulations with higher polymer content produced more compact microspheres and reduced material loss, especially in formulations using the heat stabilization technique, which minimized washing steps and loss during separation.

Swelling Index:

Swelling indices increased with increasing polymer concentration, ranging from 135.2% (F1) to 153.8% (F6). Chitosan's hydrophilic nature contributed significantly to fluid uptake in acidic environments. The higher swelling index observed in F6 suggests an enhanced mucoadhesive interaction potential and slower diffusion-controlled drug release due to the swollen gel barrier. These findings indicate a positive correlation between swelling capacity and the matrix's ability to prolong drug retention in the gastric mucosa.

Table 5: Swelling Index (% in SGF, pH 1.2):

Formulation Code	Swelling Index (%)	Swelling Behavior Observation
F1	135.2	Rapid hydration, moderately stable
F2	142.6	Progressive swelling, maintains integrity
F3	150.3	Strong matrix expansion, robust shape
F4	138.5	Moderate swelling, slight softening
F5	146.1	Stable and continuous fluid uptake
F6	153.8	Excellent swelling, retained spherical shape

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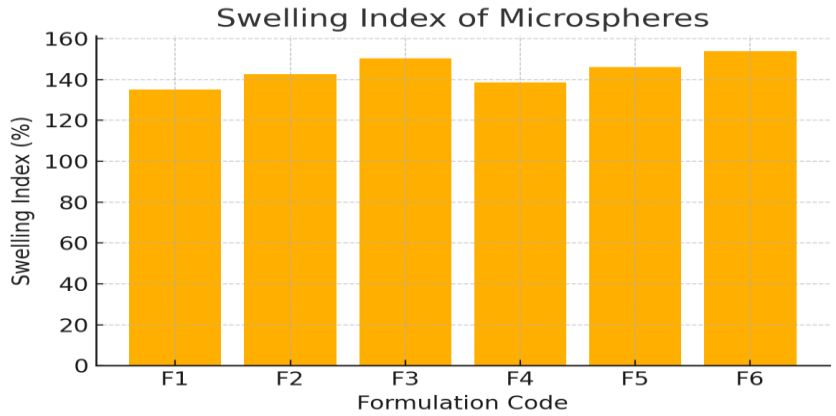

Figure 6. Swelling Index (% in SGF, pH 1.2)

Mucoadhesion Study:

Ex vivo mucoadhesion studies demonstrated a progressive enhancement in mucosal retention with increasing concentrations of chitosan in the microsphere formulations. Among the batches tested, F1 exhibited the lowest mucoadhesive strength (68.1%), while F6 showed the highest adhesion percentage (79.3%). This increase in adhesion is likely due to the higher swelling capacity of F6, which allows greater surface contact with the mucosal tissue, as well as more robust electrostatic interactions between the cationic amine groups of chitosan and the negatively charged sialic acid residues of mucin. Additionally, the larger surface area associated with higher polymer content may have further contributed to improved adherence. These findings strongly support the potential of F6 to prolong gastric residence time, thereby enhancing localized delivery of andrographolide for the effective management of *Helicobacter pylori*-associated peptic ulcers.

Table 6: Mucoadhesion Study (Ex vivo, % Adhered to Gastric Mucosa):

Formulation Code	Mucoadhesion (%)	Adhesion Strength Observation
F1	68.1	Weak interaction, minimal gastric retention
F2	72.5	Moderate mucosal adhesion
F3	76.4	Strong bioadhesive capacity
F4	70.2	Good retention using oil-hardened matrix
F5	74.9	Stable adhesion, consistent mucosal contact
F6	79.3	Highest adhesion, suitable for gastric targeting

In Vitro Drug Release Study:

In vitro release studies of andrographolide-loaded mucoadhesive microspheres demonstrated a biphasic release pattern across all formulations, characterized by an initial moderate burst release followed by a sustained release phase. The total cumulative drug release over a 12-hour period ranged from 81.6% for F1 to 91.5% for F6. Notably, formulations with higher chitosan content, such as F3 and F6, exhibited a more controlled and prolonged release, attributed to denser polymeric matrices that retard drug diffusion. Among all, F6 displayed the most sustained release profile, indicating an optimally structured microsphere matrix capable of modulating drug release kinetics efficiently. Kinetic modelling revealed that the Korsmeyer–Peppas model best described the release data, with values of the release exponent (n) suggesting a non-Fickian (anomalous) transport mechanism involving a combination of diffusion and polymer relaxation. These findings highlight the potential of F6 as a gastroretentive delivery system for the sustained therapeutic management of *H. pylori*-associated gastric ulcers.

Table 7: In Vitro Cumulative Drug Release (% w/w) of Formulations F1–F6

Time (h)	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)	F6 (%)
0.5	12.4	13.5	14.8	12.9	13.9	15.1
1.0	22.6	24.9	27.2	23.7	25.8	28.1
2.0	36.7	39.8	43.5	38.4	41.2	45.9
4.0	53.2	56.4	60.1	55.2	58.3	63.5

6.0	66.8	69.7	73.6	67.5	71.3	76.4
8.0	74.3	77.5	81.2	75.1	78.7	84.6
10.0	78.9	82.1	86.3	79.4	83.2	89.3
12.0	81.6	85.2	89.4	83.1	86.7	91.5

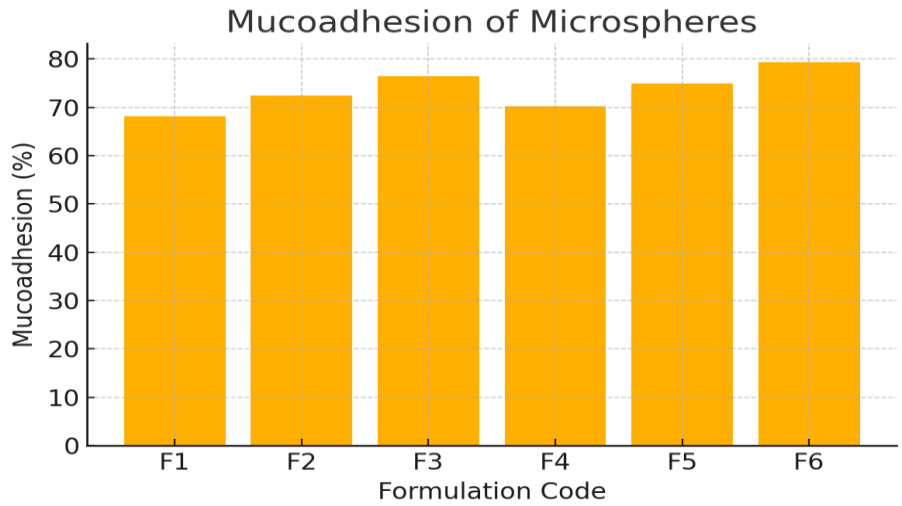

Figure 7. Mucoadhesion Study (Ex vivo, % Adhered to Gastric Mucosa)

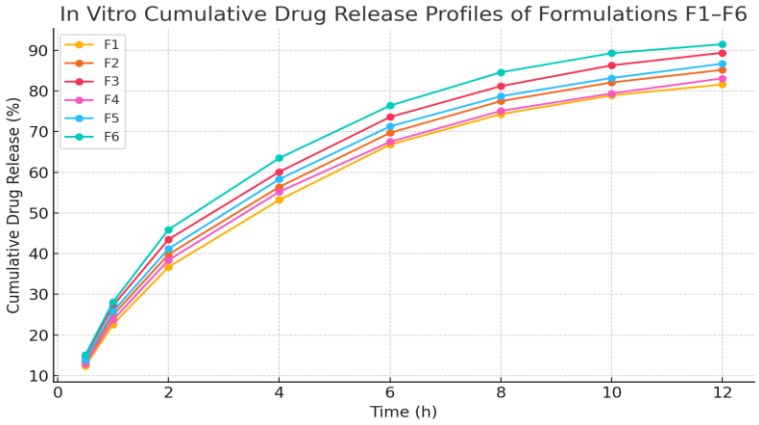

Figure 8. In Vitro Drug Release Study

CONCLUSIONS:

This study successfully demonstrated the feasibility of formulating andrographolide-loaded mucoadhesive microspheres for targeted delivery in the treatment of *H. pylori*-associated peptic ulcers. Two stabilization methods—chemical (ionic gelation using TPP) and heat (emulsion-solvent evaporation)—were employed, with chitosan as the core polymer due to its mucoadhesive and biocompatible properties. The microspheres exhibited desirable physical characteristics, including spherical morphology and particle size ranging between 48.6 µm and 58.7 µm. Entrapment efficiency was notably influenced by the polymer concentration and stabilization technique, with formulation F6 (heat stabilized, high chitosan) achieving the highest drug loading (16.5%) and entrapment efficiency (80.3%). The swelling index and mucoadhesion studies further validated the gastroretentive capability of the microspheres, critical for prolonged drug residence time at the infection site. The mucoadhesion potential increased consistently with chitosan content, reaching a peak of 79.3% in F6. In vitro release studies demonstrated sustained drug release over 12 hours, with F6 again outperforming other formulations with 91.5% cumulative release. This release behavior followed non-Fickian diffusion kinetics, indicating a combination of polymer swelling and drug diffusion mechanisms. Overall, F6 emerged as the optimal formulation, offering the most favorable balance of drug entrapment, release control, and mucoadhesive strength.

These attributes make it a strong candidate for further in vivo studies to assess pharmacodynamic performance and anti-*H. pylori* efficacy. The developed system holds the potential to improve therapeutic outcomes in peptic ulcer patients by ensuring site-specific action, minimizing drug degradation in acidic environments, and reducing dosing frequency. This formulation approach may be further extended to other plant-derived therapeutic agents intended for gastric-targeted applications.

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