Fabrication, Characterization, and Assessment of Berberine-Loaded Mucoadhesive Microspheres for Targeted Peptic Ulcer Therapy

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KEYWORDS

Mucoadhesive, Microspheres, Berberine, Chemical stabilization, Gastroretentive, Floating.

ABSTRACT:

This study focused on the development, characterization, and evaluation of mucoadhesive microspheres for sustained drug delivery using various formulations. Berberine, a bioactive compound with significant anti-inflammatory and anti-ulcer properties, served as the model drug. Microspheres were fabricated using a single-phase emulsification technique with bovine serum albumin and Carbopol 934P as polymers, and glutaraldehyde as a cross-linker. Parameters such as particle size, elongation ratio, uniformity index, and morphology were assessed, confirming the successful formation of microspheres with desirable properties. The in vitro studies demonstrated high encapsulation efficiency (up to 99.30%) and controlled drug release over 12 hours, with CSF1 showing the highest release (98.18%) at pH 1.2. Swelling and mucoadhesion studies revealed a strong correlation between hydration capacity and adhesive properties, with CSF2 exhibiting superior mucoadhesion (77.45%). Scanning electron microscopy confirmed uniform spherical morphology for most formulations. Statistical analysis validated the reproducibility of the results, with minimal variability. This comprehensive evaluation highlights the potential of these microspheres for targeted gastric drug delivery, ensuring prolonged retention and controlled release. The findings suggest that formulations like CSF1 and CSF2 are optimal for therapeutic applications requiring sustained action and robust adhesion to the gastric mucosa.

1. Introduction

Gastroretentive mucoadhesive microspheres are advanced drug delivery systems designed to prolong the retention of therapeutic agents in the stomach, ensuring localized drug release and enhanced therapeutic efficacy. These systems leverage both gastroretention and mucoadhesion mechanisms to address the challenges posed by short gastric residence times and rapid drug degradation in the gastrointestinal tract. Mucoadhesive microspheres adhere to the gastric mucosa, utilizing polymers that interact with mucin glycoproteins, thus resisting peristaltic movements and ensuring prolonged contact time at the targeted site (Khan et al., 2015, Shadab et al., 2012, Jain et al., 2012, Shivanand, 2010). The formulation of gastroretentive microspheres typically involves biodegradable polymers such as chitosan, Carbopol, and albumin, which not only provide biocompatibility but also control drug release kinetics. These systems are particularly beneficial for drugs with poor solubility, stability issues in alkaline pH, or narrow absorption windows. By remaining in the acidic gastric environment, gastroretentive microspheres enhance the solubility and bioavailability of such drugs while minimizing systemic side effects (Mohan et al., 2014, Shadab et al., 2012, Patil and Sawant, 2008).In addition, the controlled release profile achieved through this system ensures a steady therapeutic concentration of the drug over an extended period, reducing dosing frequency and improving patient compliance. These attributes make gastroretentive mucoadhesive microspheres a promising approach for managing chronic gastric disorders such as peptic ulcers (Shukla and Tiwari, 2012, Sinha et al., 2004, Sivadas et al., 2008).

Peptic ulcer disease (PUD) is a chronic gastrointestinal condition characterized by the erosion of the gastric or duodenal mucosa due to an imbalance between aggressive factors, such as gastric acid and Helicobacter pylori, and protective factors like mucus and bicarbonate. Symptoms include epigastric pain, nausea, and bleeding in severe cases, significantly impairing quality of life. Standard treatments for PUD involve acid-suppressing agents like proton pump inhibitors, antibiotics for H. pylori, and mucosal protectants. However, these therapies often suffer from limited efficacy due to poor patient adherence, rapid drug clearance, or the inability to maintain therapeutic drug levels at the ulcer site. The treatment complexity is further exacerbated by the need for combination therapies to eradicate H. pylori, which increases the risk of systemic side effects. Additionally, drugs used in PUD management often degrade in the alkaline environment of the small intestine, reducing their therapeutic potential. These challenges necessitate innovative drug delivery approaches to enhance localized drug action, improve bioavailability, and minimize systemic exposure (Chun et al., 2005a, Chun et al., 2005b, Soane et al., 1999, Sun et al., 2009, Hall, 2010).

Berberine, a natural alkaloid with potent anti-inflammatory, anti-bacterial, and anti-ulcer properties, is a promising candidate for managing peptic ulcers. However, its clinical application is hindered by poor solubility, low bioavailability, and rapid elimination. Gastroretentive mucoadhesive microspheres of berberine address these limitations by localizing drug action in the stomach, where the therapeutic effect is needed most (Tai and McAlindon, 2021, Goodman et al., 2011, N and Chopra, 2006, Khare, 2007, Mukherjee et al., 2010). The prolonged retention of berberine in the stomach through mucoadhesive microspheres ensures sustained drug release, allowing for consistent therapeutic concentrations at the ulcer site. This localized delivery reduces systemic drug exposure and associated side effects, while enhancing the efficacy of Helicobacter pylori eradication. Moreover, the controlled release profile minimizes dosing frequency, improving patient adherence to therapy (Tripathi, 2008, Brenner and Stevens, 2009). By maintaining berberine in the acidic gastric environment, gastroretentive microspheres prevent drug degradation in alkaline pH, further enhancing its stability and bioavailability. This innovative delivery system not only improves

the therapeutic potential of berberine but also offers a cost-effective and patient-friendly solution for managing peptic ulcers, addressing the limitations of current treatment regimens (Shukla and Tiwari, 2012, Sinha et al., 2004, Sivadas et al., 2008). Berberine is known for its diverse pharmacological properties, including anti-inflammatory, antifungal, antibacterial, antiviral, antidiabetic, and anti-ulcer activities. These attributes make it a promising candidate for addressing various gastrointestinal conditions. To harness its therapeutic potential for treating peptic ulcers, the current study focuses on the formulation, characterization, and evaluation of mucoadhesive microspheres loaded with berberine. These microspheres are designed to provide localized action, enhance drug retention at the ulcer site, and offer sustained release for improved therapeutic efficacy.

2. Material and Methods

Chemicals, reagents and drugs

A pure sample of berberine, used as the standard reference substance, was procured from Sigma Aldrich, Mumbai, India. The authenticated suppliers of Berberis species were identified at Azadpur Mandi, Khar Bauri, Delhi, India. Berberine was extracted in the laboratory using a previously validated and standardized extraction method to ensure purity and reproducibility. All other chemicals and reagents used in the study were of analytical grade and sourced from trusted suppliers. These included ammonium hydroxide, liquid paraffin, silica gel, hydrochloric acid, glutaraldehyde, carbopol, acetone, and bovine serum albumin (BSA), all of which were essential for the fabrication and evaluation processes.

Preparation and Formulation of microspheres: Chemical stabilization Method

Berberine-loaded mucoadhesive microspheres were formulated using a single-phase emulsification technique. Bovine serum albumin (BSA) and Carbopol 934P were employed as the polymers, while glutaraldehyde served as the chemical cross-linker. The process began with dissolving the required amounts of berberine and polymers in water to form a homogeneous solution. This solution was then introduced dropwise into liquid paraffin contained in a beaker, maintained at 15°C, and subjected to continuous stirring at 100 rpm to create the primary emulsion. To facilitate surface cross-linking, glutaraldehyde was carefully added drop by drop to the emulsion. The reaction was allowed to proceed for six hours to ensure adequate cross-linking, after which the microspheres were separated by centrifugation. The collected microspheres underwent three washes with acetone to remove impurities, followed by vacuum drying to obtain the final product. Four distinct microsphere formulations, labelled CSF1, CSF2, CSF3, and CSF4, were prepared with varying drug-to-polymer ratios of 1:1, 1:2, 1:3, and 1:4, respectively. These formulations aimed to optimize drug loading and release characteristics for enhanced therapeutic efficacy.

Preparation and Formulation of microspheres: Heat stabilization method

In the heat stabilization procedure, berberine-loaded microspheres were rigidified and stabilized by applying heat, resulting in the denaturation of surface proteins. The polymers used in the formulation were Carbopol 934P and bovine serum albumin (BSA). The process began with dissolving a predetermined amount of berberine and the polymers in water to create a homogeneous solution. This solution was then added to a beaker containing liquid paraffin, maintained at a temperature of 15°C, and subjected to constant shear at 100 rpm to form the primary emulsion. To achieve surface rigidization, the temperature of the emulsion was gradually increased in a controlled manner up to 70°C. The application of heat caused denaturation of the protein, leading to stabilization and hardening of the microsphere surfaces.

After heat stabilization, the microspheres were collected through centrifugation over a six-hour period to ensure efficient separation. The collected microspheres were then washed three times with acetone to remove residual impurities, followed by vacuum drying to obtain the final product. Using this procedure, four formulations of microspheres were prepared with varying drug-to-polymer ratios of 1:1, 1:2, 1:3, and 1:4, labelled as HSF1, HSF2, HSF3, and HSF4, respectively. These formulations were designed to evaluate the effects of polymer concentration on the drug loading, stabilization, and release properties of the microspheres.

Characterization of the Prepared Microspheres

Particle size study, Assessment of Uniformity Index and Elongation ratio

The particle size of the microspheres was meticulously measured using a stage micrometer to ensure precision and consistency in formulation. To prepare the sample for analysis, 5 mg of dry microspheres were weighed and suspended in distilled water. Ultrasonication was applied for 5 seconds to evenly disperse the particles, preventing aggregation and ensuring accurate measurement. A drop of the well-dispersed suspension was then placed on a clean glass slide, and the microspheres were counted and measured under a stage ocular micrometer. For each batch, at least 200 microspheres were analyzed to obtain a statistically significant representation of the particle size distribution. The mean particle diameter of the microspheres was calculated and expressed as the average size ± standard deviation (SD) to reflect the uniformity of the formulation process. This parameter is critical, as particle size directly influences the surface area, drug loading, and release profiles of the microspheres. In addition to particle size, the shape of the microspheres was evaluated by determining the elongation ratio (ER), defined as the quotient of the length and width of the microspheres. This ratio provided insights into the morphological characteristics of the particles. The classifications were as follows:

- Perfectly spherical shape: ER = 1
- Nearly spherical shape: $1.1 < ER \leq 1.15$
- Non-spherical shape: $ER > 1.15$

This assessment of particle morphology is essential, as the shape affects the mucoadhesive properties, drug distribution, and overall stability of the microspheres. By combining size and shape analysis, the study ensured that the fabricated microspheres met the desired specifications for optimal performance in drug delivery applications (Das and Ng, 2010). The following formula was used to get the Uniformity Index (UI):

$$UI = \frac{D_W}{D_N}$$

Additionally, Dw and Dn, which stand for weight average diameter and number average diameter, respectively, were obtained using different formulas. Values below 1.2 indicate a monodisperse distribution, whereas values above 1.2 indicate a wide particle size distribution, according to the Uniformity Index, or UI.

Scanning Electron Microscopy (SEM): **Morphological Examination**

The morphology of the microspheres was analyzed using scanning electron microscopy (SEM) to assess their surface characteristics and structural integrity. The analysis was conducted using a JSM-5310LV scanning electron microscope (Tokyo, Japan). To prepare the microspheres for imaging, they were mounted on metal stubs using double-sided adhesive tape, ensuring stability during the scanning process. Once securely attached, the samples were coated with a thin layer of gold, approximately 150 Å thick, using a sputter coater under vacuum conditions. This gold coating enhanced the conductivity of the microspheres and ensured high-resolution imaging by reducing charging effects during electron beam exposure. The prepared stubs were

then visualized under the SEM, which provided detailed images of the microspheres' surface morphology. This analysis offered critical insights into the shape, size, texture, and any surface irregularities or defects present in the microspheres. Such morphological evaluation is essential for confirming the uniformity and quality of the microspheres, which directly impact their performance in drug delivery applications (Hardenia et al., 2011).

Encapsulation Efficiency and Drug Loading

The actual drug concentration in the microspheres was determined using a UV spectrophotometer (Shimadzu UV, 1601). Samples from each batch of microspheres were dissolved in a phosphate buffer solution (pH 7.4) to ensure complete drug release and obtain a homogeneous solution suitable for analysis. The absorbance of the solution was measured at the drug's specific wavelength, and the concentration was calculated based on a pre-established calibration curve.

To evaluate the encapsulation efficiency, the ratio of the actual drug content to the theoretical drug content was calculated (Yadav and Jain, 2011). The encapsulation efficiency was then expressed as a percentage using the following formula:

Encapsulation Efficiency (%) = (Actual Drug Content/ Theoretical Drug Content) × 100

Percentage Yield

The production yield of the microspheres was determined by comparing the actual quantity of microspheres obtained to the theoretical amount expected based on the initial formulation components. This calculation provided insight into the efficiency of the microsphere fabrication process. The percentage yield (% yield) was computed using the formula: (Yadav and Jain, 2011)

$$\text{Percentage Yield (\%)} = \frac{\text{Actual Weight of Microspheres Collected}}{\text{Theoretical Weight of Microspheres}} \times 100$$

The % yield calculation helps identify material losses during the manufacturing process, such as those due to adherence to equipment or incomplete recovery. A high production yield indicates an efficient process, with minimal wastage of materials and optimal recovery of the formulated microspheres. Each batch's production yield was expressed as a mean percentage to reflect process consistency and reproducibility.

Swelling Index

The swelling behavior of the microspheres was evaluated in phosphate buffer (pH 6.8) to understand their hydration and swelling characteristics over time. The sizes of the microspheres, both in their dried state and after incubation in the phosphate buffer for specified time intervals (0.3, 1.0, 3.0, and 5.0 hours), were measured using a microscopic method. The swelling percentage at each time interval was calculated by comparing the diameter of the microspheres at a given time t (Dt) to their initial diameter at t=0(D0). The swelling index was determined using the following equation (Shivanand et al., 2010).

:

$$\textbf{Swelling Index} = \frac{We - W_O}{W_O}$$

Where Wo denotes the dry microspheres' initial weight and We denotes the bigger, swollen microspheres' weight in the medium at equilibrium. This analysis provided valuable information about the hydration capacity and swelling kinetics of the microspheres, which are critical for understanding their mucoadhesive properties and drug release behavior in the gastrointestinal environment. The swelling percentage was reported for each time point, allowing for an evaluation of the time-dependent swelling profile of the microspheres.

Mucoadhesion study

The mucoadhesive properties of the microspheres were evaluated using the in vitro "wash-off method," a widely recognized technique to assess the adhesive strength of formulations on biological tissues. A 1x1 cm piece of goat stomach mucosa was carefully prepared and secured onto a glass slide using thread to ensure stability during the test. The tissue sample was first rinsed with water to remove any debris and moisten the surface, mimicking the natural conditions of gastric mucosa. Approximately 100 microspheres were then evenly spread over the surface of the prepared mucosal tissue. The glass slide with the adhered microspheres was placed in one of the grooves of a USP disintegration apparatus. The apparatus was filled with simulated gastric fluid (pH 1.2) to replicate the gastric environment. The disintegration apparatus was operated to move the tissue sample in an up-and-down motion within the fluid, simulating the mechanical forces experienced in the stomach. At specified intervals—30 minutes, 1 hour, and then hourly for up to 4 hours—the number of microspheres remaining adhered to the mucosal tissue was counted. This provided a quantitative measure of the mucoadhesive strength of the microspheres over time, reflecting their potential to remain attached to the gastric mucosa in vivo. The results were analyzed to determine the retention efficiency of the microspheres, which is critical for ensuring prolonged gastric residence time and effective drug delivery. This method helped to identify the optimal formulation with the highest mucoadhesive capability, suitable for sustained therapeutic action in the gastrointestinal tract (Hardenia et al., 2011).

$$\text{Percent mucoadhesion} = \frac{\text{weight of adhered microspheres}}{\text{weight of applied microspheres}} \times 100$$

In vitro drug release study

The in vitro drug release study was performed to evaluate the release profile of the drug from the formulated microspheres under simulated gastric conditions. The experiment utilized a USP paddle apparatus as the dissolution equipment, with 900 mL of 0.1N HCl serving as the dissolution medium. The study was conducted at a controlled temperature of 37 ± 0.5°C and a paddle rotation speed of 100 RPM to mimic the physiological conditions of the stomach. At predetermined time intervals, 5 mL aliquots of the dissolution medium were withdrawn for analysis, ensuring precise sampling. To maintain sink conditions, each withdrawn aliquot was replaced with an equal volume of fresh dissolution medium. The samples were then analyzed using a UV spectrophotometer at a wavelength of 250 nm, specific to the drug, to determine the drug concentration in the medium. The percent cumulative drug release was calculated for each time point, providing insights into the release kinetics and the sustained release potential of the microspheres. This step-by-step approach ensured accurate and reproducible data, enabling the evaluation of how effectively the formulation delivers the drug over time in conditions mimicking the gastric environment. The drug release profiles were further analysed to determine the release mechanism, such as diffusion-controlled or erosion-based release, which is critical for optimizing the therapeutic performance of the microspheres (Shivanand et al., 2010).

Statistical analysis

Statistical analysis was performed to validate the reliability and significance of the experimental data. All results were expressed as mean ± standard deviation (SD) and derived from at least three independent experiments. One-way analysis of variance (ANOVA) was employed to compare differences between multiple formulations, followed by post hoc tests to identify specific group differences. A p-value of less than 0.05 was considered statistically significant. Data analysis was carried out using statistical software to ensure accuracy and

consistency. Correlation coefficients were calculated for drug release kinetics, while regression analysis was used to evaluate the best-fit kinetic models. These analyses ensured that the observed variations and trends in the data were scientifically robust and reproducible

3. Results and Discussion

Particle sizes, Elongation ratio, Uniformity index and microspheres shape

The analysis of the particle size, elongation ratio (ER), uniformity index (UI), and shape of the microspheres presented in Table 1 highlights significant differences among the formulations. The particle size of the microspheres ranged from 4.65 µm (HSF1) to 9.01 µm (HSF4), with varying degrees of standard deviation, indicating consistency in the formulation process. CSF1 and CSF4 exhibited relatively larger particle sizes among the CSF formulations, while HSF4 had the largest particle size overall. The elongation ratio (ER) varied between 1.281 (HSF4) and 1.451 (CSF1), providing insights into the morphological characteristics of the microspheres. Microspheres with ER closer to 1.1, such as HSF4 and HSF2, were classified as spherical, while those with higher ER values, such as CSF1 and HSF1, displayed deviations from spherical shapes, making them non-spherical. The uniformity index (UI) values ranged widely from 0.921 (CSF4) to 1.798 (HSF1). Higher UI values, as seen in HSF1, suggest less uniformity in particle size distribution, while lower UI values, such as in CSF4, indicate a more uniform size distribution. Shape classification further supported this, with spherical microspheres demonstrating better uniformity and lower ER values, contributing to enhanced aerodynamic and mucoadhesive properties. Overall, spherical formulations like CSF4 and HSF4 demonstrated optimal characteristics, such as lower ER and higher uniformity, which are favorable for consistent drug release and effective mucoadhesion. Non-spherical microspheres, while less uniform, may offer advantages in surface interactions depending on their application. These findings provide a comparative basis for selecting formulations based on desired performance outcomes in drug delivery systems.

Table 1. Particle sizes, Elongation ratio, Uniformity index and microspheres shape

Formulation	Particle size (µm) ± SD	UI	ER	Shape
CSF1	8.12 ± 0.15	1.241	1.451 ± 0.13	Spherical
CSF2	7.17 ± 0.17	1.1007	1.301 ± 0.13	Spherical
CSF3	6.16 ± 0.17	1.2196	1.401 ± 0.19	Non spherical
CSF4	7.99 ± 0.19	0.921	1.341 ± 0.18	Spherical
HSF1	4.65 ± 0.13	1.798	1.431 ± 0.10	Non spherical
HSF2	7.82 ± 0.19	1.025	1.311 ± 0.09	Spherical
HSF3	5.67 ± 0.15	0.991	1.331 ± 0.12	Non spherical
HSF4	9.01 ± 0.12	0.9934	1.281 ± 0.09	Spherical

Where UI= Uniformity index and ER= Elongation ratio

Preparation of microspheres: Percentage yield

The percentage yield data in Table 2 reflects the efficiency of microsphere formulation across different batches. The yields ranged from 94.849% (HSF3) to 98.319% (CSF2), indicating minimal material loss during the fabrication process. Among the CSF formulations, CSF2 exhibited the highest yield (98.319 ± 1.24%), suggesting an optimized preparation process with minimal waste. Conversely, CSF3 had the lowest yield (95.329 ± 0.90%) among the CSF group, potentially due to minor variations in processing parameters. Similarly, within the HSF formulations, HSF2 achieved a relatively higher yield (96.949 ± 1.24%), while HSF3 and HSF4 showed slightly lower yields, both hovering around 94.9%. These variations, though minor,

may reflect differences in polymer-drug interactions or recovery efficiency during microsphere collection. Overall, the high yields across all formulations indicate that the fabrication process is robust and well-controlled. The slight variations in percentage yield could be attributed to batch-specific factors such as polymer consistency, cross-linking efficiency, or procedural differences. These results underline the reproducibility and reliability of the formulation techniques employed, with all batches achieving yields suitable for large-scale production. Formulations with higher yields, like CSF2 and HSF2, may be preferred for their efficient use of materials and potential cost-effectiveness in scale-up processes.

Table 2. Percentage yield.

Formulation	**Percentage yield**
CSF1	97.639 ± 0.96
CSF2	98.319 ± 1.24
CSF3	95.329 ± 0.90
CSF4	95.649 ± 1.16
HSF1	96.659 ± 1.09
HSF2	96.949 ± 1.24
HSF3	94.849 ± 1.14
HSF4	94.949 ± 1.08

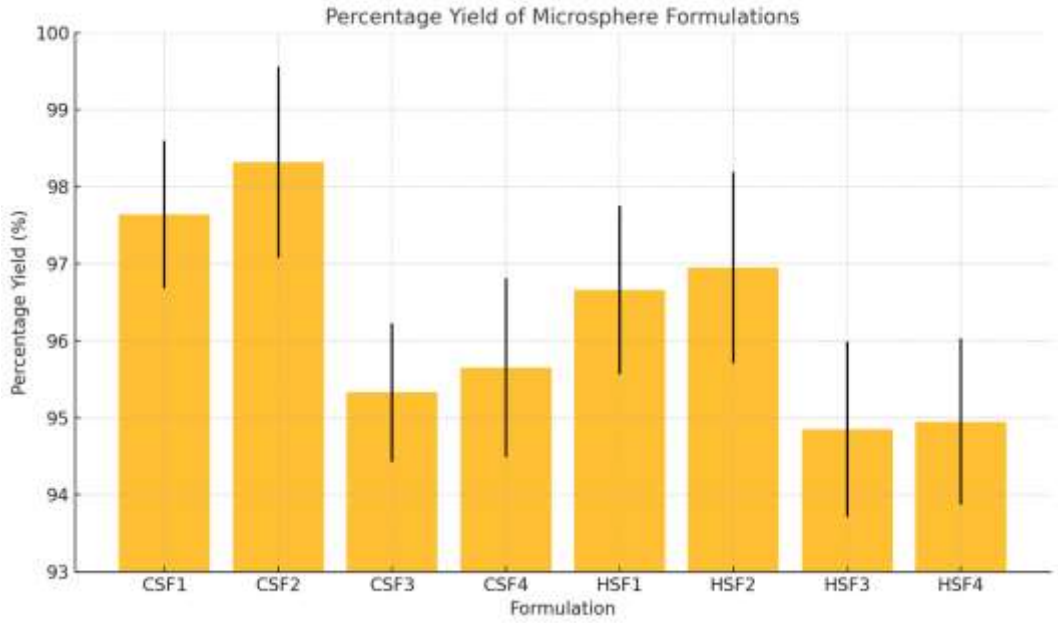

Figure 1. Percentage yield

Encapsulation efficiency, and Drug loading of microspheres

The encapsulation efficiency (EE) and loading capacity (LC) data presented in Table 3 provide insights into the effectiveness of drug incorporation and the amount of drug loaded into the microspheres for different formulations. The encapsulation efficiency values ranged from 95.70% (HSF3) to 99.30% (CSF2), indicating that the microsphere fabrication process was highly effective across all formulations. CSF2 exhibited the highest EE (99.30%), reflecting minimal drug loss during formulation. Conversely, HSF3 demonstrated slightly lower EE, which could be due to differences in polymer-drug interactions or variations in cross-linking efficiency. Loading capacity, a measure of the drug content per unit weight of microspheres,

varied significantly among formulations. The highest LC was observed in HSF1 (54.41%), followed closely by CSF2 (52.44%), suggesting efficient utilization of the polymer matrix in these formulations. In contrast, HSF3 had the lowest LC (28.73%), which may impact its drug release potential and overall therapeutic efficacy. Formulations such as CSF2 and HSF1, with high EE and LC, are optimal for achieving sustained drug release and enhanced therapeutic performance. The observed variations between formulations underscore the influence of polymer concentration, drug-to-polymer ratio, and preparation techniques on the encapsulation and loading outcomes.

Table 3. Encapsulation efficiency and Loading capacity

Formulation	**EE**	**LC**
CSF1	98.43	52.21
CSF2	99.30	52.44
CSF3	96.21	35.43
CSF4	96.43	36.85
HSF1	97.49	54.41
HSF2	97.67	51.63
HSF3	95.70	28.73
HSF4	95.79	34.74

Where EE= Encapsulation efficiency, LC= Loading capacity

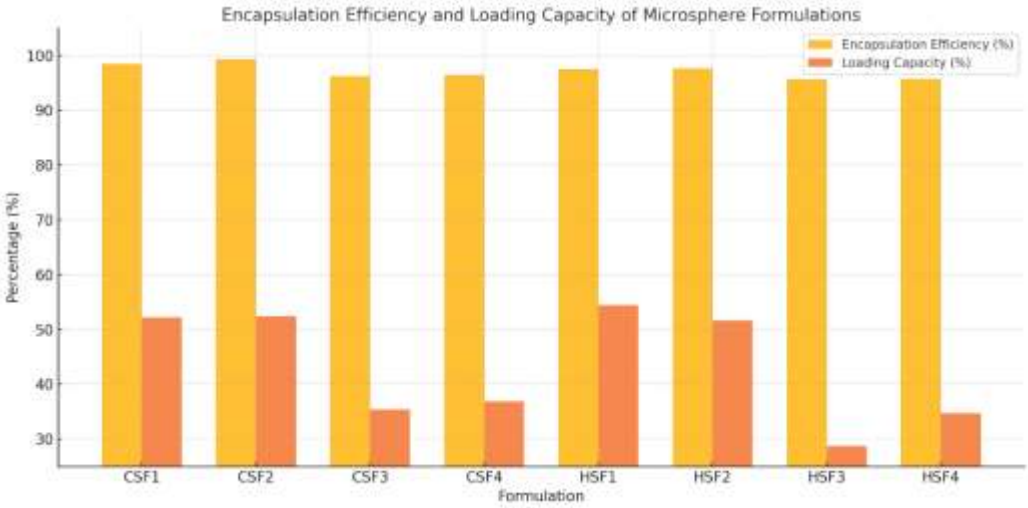

Figure 2. Encapsulation efficiency and Loading capacity

Scanning Electron Microscopy (SEM)

The Scanning Electron Microscopy (SEM) analysis provided detailed insights into the surface morphology and structural characteristics of the microspheres. The SEM images revealed that the majority of the formulations exhibited a smooth, spherical surface, indicating uniform polymer distribution and effective encapsulation of the drug. Formulations classified as spherical, such as CSF1, CSF2, and HSF4, demonstrated consistent shape and surface integrity, which are critical for controlled drug release and mucoadhesion. Non-spherical formulations, including HSF1 and CSF3, displayed slight surface irregularities and asymmetry, possibly due to variations in polymer concentration or processing conditions. These morphological differences could influence drug release kinetics and mucoadhesive properties. Overall, SEM analysis confirmed the successful fabrication of microspheres with desirable structural attributes for sustained drug delivery applications.

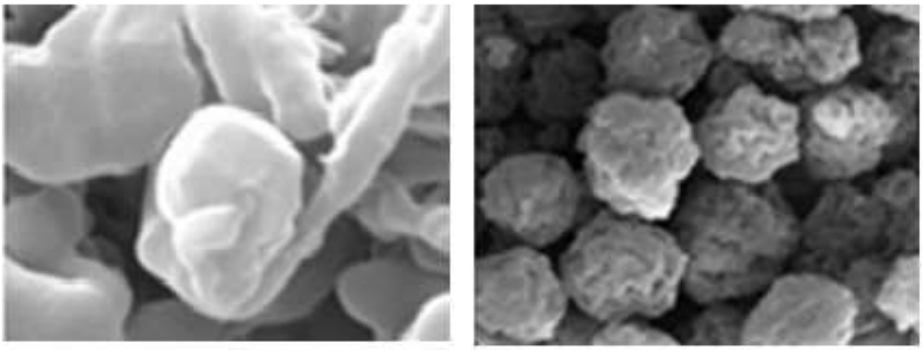

Figure 3. SEM photomicrograph of the formulation of the microsphere (CSF)

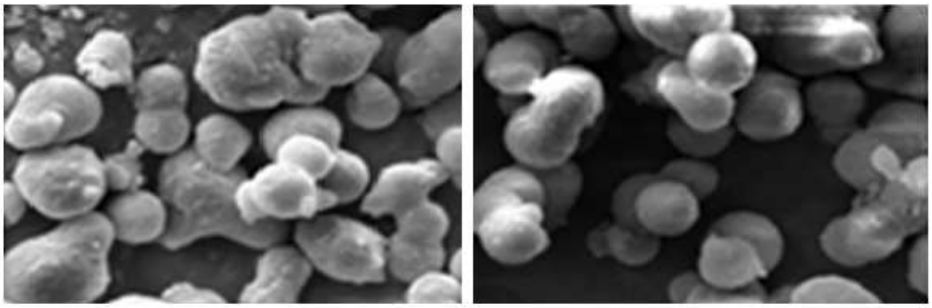

Figure 4. SEM photomicrograph of the formulation of the microsphere (HSF)

Swelling Index and Mucoadhesioon

The swelling index and percentage mucoadhesion after 600 minutes provide key insights into the performance of the microsphere formulations, particularly their capacity to retain moisture and adhere to mucosal surfaces. The swelling index (SI) values ranged from 0.20 (CSF4, HSF4) to 0.99 (CSF1, HSF1), indicating significant differences in hydration capacity. Formulations with higher swelling indices, such as CSF1, CSF3, and HSF1, showed greater water uptake, which could enhance their mucoadhesive properties by increasing contact time with the mucosal surface. Conversely, formulations with lower SI values, such as CSF4 and HSF4, might exhibit faster erosion or reduced mucoadhesion due to limited swelling. The percentage mucoadhesion after 600 minutes varied from 50.29% (HSF4) to 77.45% (CSF2). CSF2 demonstrated the highest mucoadhesion, indicating robust interaction with the mucosal surface despite a relatively low swelling index. This suggests that factors other than swelling, such as polymer composition and surface properties, significantly influence adhesion. On the other hand, formulations like HSF4 and HSF2, which exhibited both low swelling and low mucoadhesion, may require optimization for improved performance. The relationship between swelling and mucoadhesion underscores the importance of balancing hydration capacity and adhesive strength to achieve optimal results. Formulations like CSF1 and HSF1, which combined high swelling indices with moderate mucoadhesion, could provide sustained drug delivery by maintaining prolonged contact with the mucosal surface.

Table 4. Index of swelling and Mucoadhesion percentage

Formulation	**Swelling index**	**Percentage Mucoadhesion after 600 min**
CSF1	0.99 ± 0.031	65.63 ± 1.52
CSF2	0.26 ± 0.011	77.45 ± 1.82
CSF3	0.98 ± 0.021	71.30 ± 1.42

CSF4	0.20 ± 0.021	57.39 ± 1.34
HSF1	0.99 ± 0.011	68.14 ± 2.56
HSF2	0.24 ± 0.011	55.42 ± 1.33
HSF3	0.98 ± 0.021	61.29 ± 1.27
HSF4	0.20 ± 0.021	50.29 ± 1.13

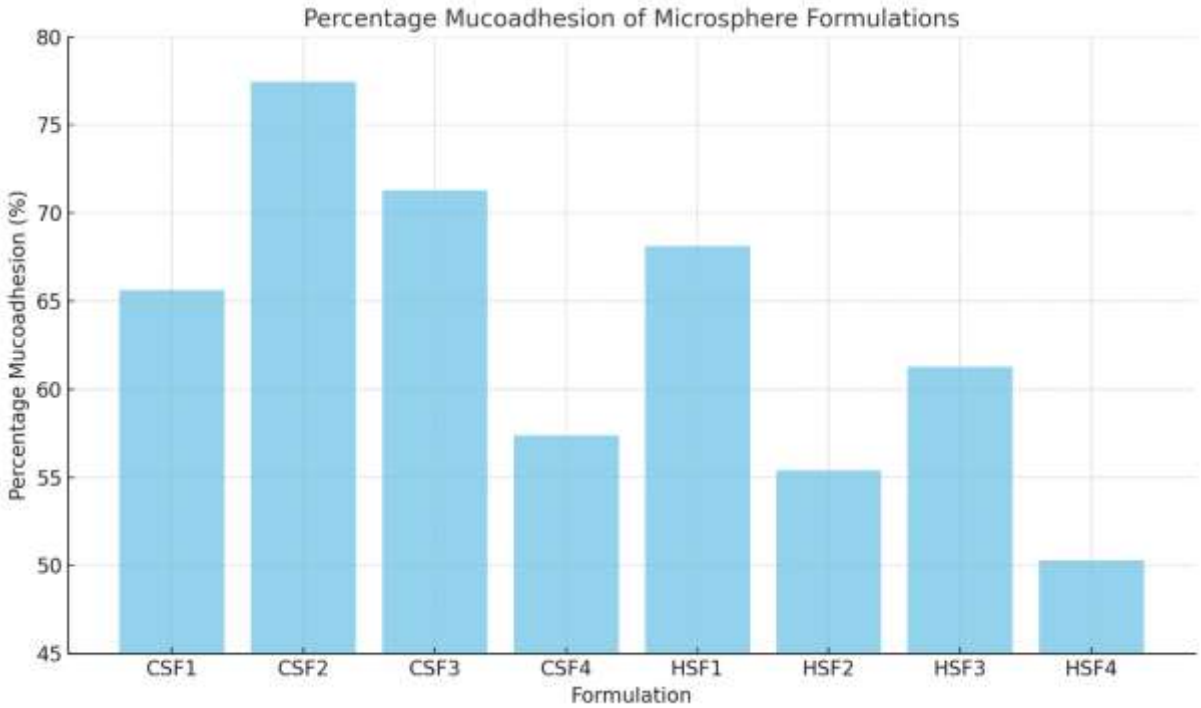

Figure 5. Mucoadhesion percentage after 600 minutes

In vitro drug release study

The in vitro drug release study at pH 1.2 highlights distinct release profiles for the various microsphere formulations over a 12-hour period. The cumulative release values suggest that all formulations achieved sustained drug release, with variations influenced by their composition and structural properties. CSF formulations generally exhibited higher cumulative drug release percentages compared to HSF formulations. Notably, CSF1 showed the highest drug release (98.18 ± 1.33%) at 12 hours, followed closely by CSF2 (96.18 ± 1.33%). These formulations maintained consistent release rates throughout the study, with significant release observed as early as the first hour (18.81 ± 1.10% for CSF1). The gradual and controlled release can be attributed to the optimized polymer-drug interactions in these formulations. Among the HSF formulations, HSF1 demonstrated the highest drug release (91.38 ± 1.46%) at 12 hours, indicating effective sustained release, albeit slightly lower than the CSF group. HSF4 exhibited the slowest release profile, reaching 89.38 ± 1.33% at 12 hours, and had the lowest release at earlier time points as well (11.06 ± 0.99% at 1 hour). This indicates a more delayed release pattern, possibly due to differences in polymer concentration or microsphere morphology. Overall, the data suggest that CSF1 and CSF2 are optimal for achieving maximum drug release over 12 hours, making them suitable for applications requiring prolonged therapeutic action. The HSF formulations, while slightly slower in release, may provide benefits for conditions where delayed release is advantageous.

Table 5. Drug release in vitro at pH 1.2

Formulation	**CSF1**	**CSF2**	**CS 3**	**CSF4**	**HSF1**	**SF 2**	**HSF3**	**HSF4**
1hr	18.81 ± 1.10	17.04 ± 1.03	16.03 ± 1.06	15.67 ± 0.90	17.02 ± 1.10	15.51 ± 1.10	14.38 ± 1.10	11.06 ± 0.99
2hr	30.42 ± 1.13	28.82 ± 1.11	27.69 ± 1.15	25.92 ± 1.24	27.70 ± 1.20	26.15 ± 1.13	25.14 ± 1.11	20.87 ± 1.11
3hr	35.15 ± 1.23	33.14 ± 1.13	31.37 ± 1.33	31.03 ± 1.13	32.37 ± 1.15	32.15 ± 1.13	30.82 ± 1.15	28.93 ± 1.17
4hr	38.15 ± 1.33	37.67 ± 1.13	37.14 ± 1.13	35.82 ± 1.33	35.37 ± 1.33	35.15 ± 1.28	33.04 ± 1.33	31.14 ± 1.33
5hr	47.57 ± 1.46	46.14 ± 1.28	40.55 ± 1.33	39.15 ± 1.33	45.19 ± 1.28	40.62 ± 1.33	38.65 ± 1.13	37.84 ± 1.46
6hr	56.14 ± 1.28	55.12 ± 1.33	53.02 ± 1.28	48.80 ± 1.13	50.91 ± 1.33	50.18 ± 1.28	49.15 ± 1.33	41.15 ± 1.33
7hr	61.13 ± 1.33	60.47 ± 1.28	60.02 ± 1.33	58.83 ± 1.33	57.14 ± 1.28	56.02 ± 1.33	54.57 ± 1.46	51.13 ± 1.26
8hr	65.71 ± 1.46	65.27 ± 1.46	65.15 ± 1.28	62.37 ± 1.28	63.09 ± 1.33	60.46 ± 1.46	60.13 ± 1.33	59.73 ± 1.33
9hr	71.15 ± 1.28	69.15 ± 1.33	68.27 ± 1.33	67.71 ± 1.33	68.57 ± 1.33	66.82 ± 1.33	65.15 ± 1.28	60.49 ± 1.46
10hr	78.82 ± 1.33	77.81 ± 1.28	74.62 ± 1.46	73.95 ± 1.46	77.04 ± 1.46	75.15 ± 1.46	73.06 ± 1.33	70.37 ± 1.33
11hr	87.18 ± 1.28	85.19 ± 1.46	83.74 ± 1.33	83.28 ± 1.33	85.37 ± 1.33	82.37 ± 1.33	81.09 ± 1.46	80.03 ± 1.46
12hr	98.18 ± 1.33	96.18 ± 1.33	94.27 ± 1.28	92.28 ± 1.46	91.38 ± 1.46	90.92 ± 1.28	90.29 ± 1.33	89.38 ± 1.33

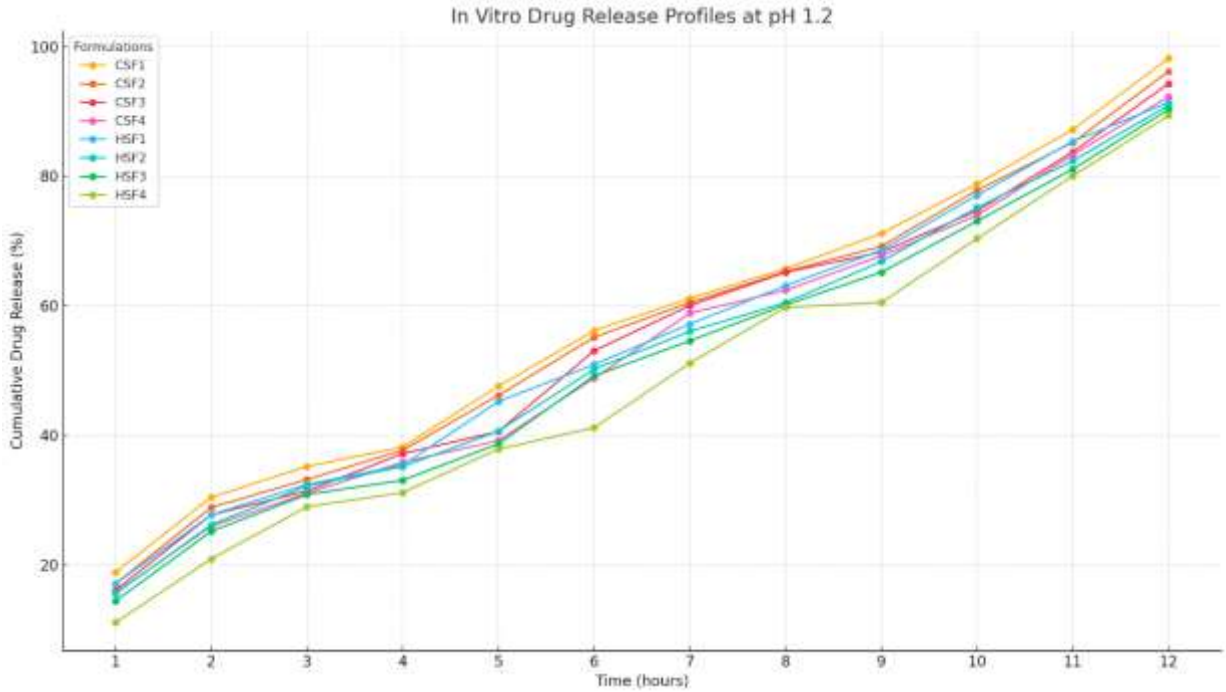

Figure 6. Drug release in vitro at pH 1.2

4. Conclusion

The study successfully formulated and evaluated mucoadhesive microspheres of berberine for potential use in gastric drug delivery. Employing bovine serum albumin and Carbopol 934P as polymers, the formulations demonstrated high encapsulation efficiencies, controlled release properties, and significant mucoadhesive strength. Among the formulations, CSF1 and CSF2 emerged as optimal, showcasing superior drug release and mucoadhesion. CSF1 exhibited the highest cumulative drug release of 98.18% at 12 hours, making it ideal for sustained drug delivery applications. Morphological studies through SEM confirmed the formation of spherical microspheres with uniform surface characteristics. Swelling and mucoadhesion tests underscored the importance of polymer concentration and microsphere morphology in enhancing adhesive properties and hydration capacity. The in vitro drug release profiles further validated the suitability of these microspheres for controlled release in gastric environments, with statistical analysis confirming the reproducibility and consistency of the formulations. These findings highlight the potential of berberine-loaded mucoadhesive microspheres as an effective system for the treatment of gastric disorders such as peptic ulcers. Future studies could explore in vivo applications and scale-up processes to facilitate clinical translation. This work sets the foundation for the development of advanced drug delivery systems targeting gastric conditions.

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