Novel Mucoadhesive Strategy for Peptic Ulcer Disease: Formulating Deglycyrrhizinated Licorice into Gastroretentive Microspheres to Prolong Gastric Residence

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ABSTRACT

This study focused on the development and evaluation of Deglycyrrhizinated Licorice (DGL)-loaded mucoadhesive microspheres using chemical and heat stabilization methods. The microspheres were prepared with varying drug-to-polymer ratios (1:1, 1:2, 1:3, and 1:4) to assess the impact of formulation parameters on encapsulation efficiency, swelling behavior, mucoadhesion, and drug release profiles. Chemical stabilization, employing glutaraldehyde as a cross-linking agent, produced formulations with superior physicochemical properties. Among the tested formulations, MFC1 emerged as the most optimized, exhibiting a high encapsulation efficiency (97.56%), favorable swelling index (0.98), and significant mucoadhesion (66.72%). In vitro drug release studies at pH 1.2 demonstrated its sustained release potential, achieving complete drug release (100.25%) over 12 hours. Scanning electron microscopy revealed a smooth, spherical morphology, contributing to uniform release kinetics. The results indicate that the chemical stabilization method is a robust approach for producing effective mucoadhesive microspheres, with MFC1 showing exceptional promise for gastric-targeted drug delivery. This work provides valuable insights into the formulation and characterization of sustained-release systems, offering a foundation for future advancements in drug delivery technologies.

Keywords: Mucoadhesive, Microspheres, DGL, Chemical stabilization, Gastroretentive, Floating.

INTRODUCTION

Mucoadhesive drug delivery systems have garnered significant attention in pharmaceutical research due to their potential to enhance therapeutic efficacy and patient compliance. These systems leverage bioadhesive polymers that interact with mucosal surfaces, prolonging drug retention at the site of action or absorption. By increasing contact time, mucoadhesive systems improve localized drug delivery and systemic absorption while minimizing drug loss. Such systems are particularly advantageous in targeting mucosal tissues, including those in the gastrointestinal tract, where sustained-release formulations can overcome challenges posed by rapid transit times and variable pH environments [1-4].

Microspheres, as a form of particulate drug delivery systems, provide unique advantages, including precise drug release profiles, improved bioavailability, and reduced dosing frequency. Encapsulation of drugs within microspheres protects the active ingredient from degradation while ensuring controlled release. The integration of mucoadhesive properties into microspheres further enhances their utility, enabling localized drug delivery with extended retention times. This dual functionality makes mucoadhesive microspheres a promising platform for various therapeutic applications, particularly for drugs requiring sustained delivery in acidic environments [2, 5, 6].

Peptic ulcer disease (PUD) is a prevalent gastrointestinal disorder characterized by lesions in the mucosal lining of the stomach or duodenum, primarily caused by an imbalance between protective mucosal factors and aggressive agents like gastric acid and pepsin. Common etiological factors include Helicobacter pylori infection, prolonged use of non-steroidal anti-inflammatory drugs (NSAIDs), stress, and lifestyle factors such as smoking and excessive alcohol consumption. Conventional treatments for PUD include proton pump inhibitors, H2 receptor antagonists, and antacids, often combined with antibiotics for eradicating *H. pylori*. However, these therapies may be associated with adverse effects, poor patient compliance, and recurrence of ulcers, necessitating the exploration of alternative therapeutic strategies [7-11].

Deglycyrrhizinated Licorice (DGL) offers a natural and promising approach for managing PUD due to its proven anti-inflammatory, cytoprotective, and wound-healing properties. Despite its therapeutic potential, the short gastric residence time and rapid degradation in the acidic environment limit its efficacy [12-14]. Rationalizing these limitations, this study focuses on formulating DGL-loaded mucoadhesive microspheres to address these challenges. By leveraging the benefits of mucoadhesive drug delivery systems, the work aims to enhance the gastric retention and controlled release of DGL, ensuring localized drug delivery and prolonged therapeutic action. This approach aligns with the need for safer, more effective, and patient-friendly alternatives for managing peptic ulcers [12-14].

Deglycyrrhizinated Licorice (DGL), a well-known herbal derivative, exhibits therapeutic potential for gastric ailments due to its anti-inflammatory, mucoprotective, and healing properties [15]. However, its efficacy is limited by rapid clearance and poor gastric retention. Incorporating DGL into mucoadhesive microspheres offers a strategic approach to address these challenges, ensuring prolonged gastric retention and sustained drug release. Formulation techniques, such as chemical and heat stabilization, play a crucial role in optimizing the

structural and functional properties of microspheres. These methods influence key parameters like encapsulation efficiency, swelling behavior, mucoadhesion, and drug release kinetics, which are critical for achieving the desired therapeutic outcomes [16-18].

The aim of this study was to develop and evaluate DGL-loaded mucoadhesive microspheres using chemical and heat stabilization methods. The objectives included formulating microspheres with varying drug-to-polymer ratios, characterizing their physicochemical properties, and assessing their swelling index, mucoadhesion, and in vitro drug release profiles. This comprehensive investigation sought to identify the most effective formulation and method, providing a foundation for advanced gastric-targeted drug delivery systems.

MATERIAL AND METHODS

Chemicals, reagents and drugs

A pure sample of Deglycyrrhizinated Licorice (DGL), which served as the standard reference substance, was sourced from Herbal Gen, Mumbai, India. This ensured the authenticity and quality required for the research. In addition to DGL, a variety of analytical-grade chemicals and reagents were employed to facilitate various experimental processes. These reagents were carefully selected from trusted suppliers to maintain consistency and reliability throughout the study. The chemicals included ammonium hydroxide, commonly used for adjusting pH and maintaining the alkaline environment necessary for specific reactions, and liquid paraffin, which played a crucial role as a hydrophobic medium in certain formulations. Silica gel was employed for its adsorbent properties, crucial in purification or chromatographic procedures, while hydrochloric acid served as a standard acid for pH adjustment and reaction facilitation. Glutaraldehyde, a bifunctional cross-linking agent, was utilized for stabilizing biomolecules or in fabrication processes requiring covalent bonding. Carbopol, a well-known polymer, was incorporated for its gel-forming and viscosity-enhancing properties, making it essential for formulating stable topical applications. Acetone, a widely used organic solvent, was employed for cleaning, extraction, or other solvent-based processes. Lastly, bovine serum albumin (BSA) was an essential reagent, often used as a protein standard in biochemical assays or as a stabilizing agent in experimental setups. Together, these high-quality reagents and chemicals were integral to the fabrication, testing, and evaluation processes of the study, ensuring reproducibility and reliability in experimental outcomes.

Preparation and Formulation of microspheres: *Chemical stabilization Method*

DGL-loaded mucoadhesive microspheres were prepared using a single-phase emulsification method. Bovine serum albumin (BSA) and Carbopol 934P were utilized as polymeric materials, while glutaraldehyde acted as the cross-linking agent. The formulation process began with dissolving the appropriate quantities of DGL and polymers in water to create a uniform solution. This solution was then introduced dropwise into liquid paraffin contained in a beaker maintained at a temperature of 15°C, with continuous stirring at 100 rpm to form the primary emulsion. To initiate surface cross-linking, glutaraldehyde was carefully added dropwise to the emulsion. The cross-linking reaction was allowed to proceed for six hours, ensuring sufficient

stabilization of the microspheres. Following the reaction, the microspheres were separated by centrifugation. The collected microspheres were washed three times with acetone to eliminate any residual impurities and then dried under vacuum to obtain the final product. Four different microsphere formulations, designated as MFC1, MFC2, MFC3, and MFC4, were developed, with drug-to-polymer ratios of 1:1, 1:2, 1:3, and 1:4, respectively. These variations were designed to evaluate and optimize drug loading capacity and release profiles, with the goal of achieving improved therapeutic performance.

Preparation and Formulation of microspheres: Heat stabilization method

In the heat stabilization method, DGL-loaded microspheres were hardened and stabilized by the controlled application of heat, resulting in the denaturation of surface proteins. The polymers utilized in this formulation included Carbopol 934P and bovine serum albumin (BSA). The process began by dissolving a specific amount of DGL and the selected polymers in water to form a uniform solution. This prepared solution was then introduced into a beaker containing liquid paraffin maintained at 15°C, with continuous stirring at 100 rpm to generate the primary emulsion. To rigidify the microspheres, the emulsion was gradually heated in a controlled manner up to 70°C. The applied heat caused the denaturation of the protein component, leading to the stabilization and hardening of the microsphere surfaces. Following the heat stabilization process, the microspheres were separated via centrifugation over a six-hour period to ensure effective recovery. The isolated microspheres were then thoroughly washed three times with acetone to eliminate any remaining impurities and subsequently dried under vacuum conditions to obtain the final product. This procedure yielded four distinct microsphere formulations, designated as MFH1, MFH2, MFH3, and MFH4, which were prepared with varying drug-to-polymer ratios of 1:1, 1:2, 1:3, and 1:4, respectively. These formulations were designed to investigate the influence of polymer concentration on critical parameters such as drug loading capacity, microsphere stabilization, and drug release characteristics.

Characterization of the Prepared Microspheres

Particle size study, Assessment of Uniformity Index and Elongation ratio

The particle size of the prepared microspheres was determined using an optical microscope equipped with a calibrated stage micrometer. A small quantity of microspheres was dispersed in a drop of glycerol or a similar dispersing medium on a clean glass slide. The slide was covered with a coverslip, ensuring no air bubbles, and examined under the microscope. At least 100 microspheres were randomly selected, and their diameters were measured using a calibrated ocular micrometer. The mean particle size was calculated along with the standard deviation to assess the uniformity of the microsphere size distribution.

The uniformity index (UI) was evaluated to determine the consistency of particle size distribution. The following formula was used to get the Uniformity Index (UI):[19].

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

Furthermore, distinct formulas were used to determine Dw and Dn, which stand for weight average diameter and number average diameter, respectively. According to the Uniformity

Index, or UI, values above 1.2 suggest a wide particle size distribution, whereas values below 1.2 indicate a monodisperse distribution.

The elongation ratio (ER) was determined to assess the shape uniformity of the microspheres. Microspheres were imaged using an optical or scanning electron microscope. The elongation ratio was calculated using the formula:

$$ER = Length/Width$$

Here, the length refers to the longest axis of the microsphere, and width refers to the shortest axis perpendicular to the length. A perfectly spherical particle would have an elongation ratio close to 1, while deviations indicate an irregular shape. Measurements were performed on at least 100 microspheres to obtain a representative value for the batch. This assessment provided insights into the morphological uniformity and potential influence on microsphere performance.

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 [20].

Encapsulation Efficiency and Drug Loading

The encapsulation efficiency and drug loading of the microspheres were determined to assess the drug incorporation and capacity of the prepared formulations. To evaluate encapsulation efficiency, a known quantity of microspheres, typically 50 mg, was dispersed in a suitable solvent, such as phosphate buffer or a mixture of organic solvents, to release the encapsulated drug. The solution was then filtered or centrifuged to separate the drug-containing supernatant from the microsphere matrix. The amount of drug present in the filtrate was quantified using an appropriate analytical method, such as UV-Visible spectroscopy, HPLC, or LC-MS, at the characteristic wavelength (λmax\lambda_{max}λmax) of the drug [21]. Encapsulation efficiency was calculated as the percentage of the drug encapsulated relative to the total drug initially used in the formulation, using the formula:

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

Similarly, drug loading was assessed to determine the amount of drug incorporated per unit weight of the microspheres. For this, the amount of drug released from a known weight of

microspheres was quantified following the same procedure as described for encapsulation efficiency. Drug loading was calculated using the formula:

DL (%)=(Weight of microspheres/Amount of drug encapsulated)×100

Both measurements were conducted in triplicate to ensure accuracy and reliability. A calibration curve of the drug in the chosen solvent was prepared to accurately determine drug concentrations. These evaluations provided vital insights into the formulation's efficiency and its potential for delivering drugs effectively.

Percentage Yield

The percentage yield of the microspheres was calculated to evaluate the efficiency of the fabrication process. After the microsphere preparation and drying, the total weight of the obtained microspheres was recorded. The percentage yield was determined by comparing the actual weight of the recovered microspheres to the total theoretical weight of all solid components used in the formulation, including the drug and polymers. The percentage yield (% yield) was computed using the formula: [21]

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

This assessment was performed for all formulations to ensure the reproducibility and efficiency of the preparation method. High percentage yield values indicated minimal loss during the formulation process, thereby reflecting an optimized technique for microsphere fabrication. All experiments were carried out in triplicate to ensure the accuracy and consistency of the results.

Swelling Index

The swelling index of the microspheres was determined to evaluate their ability to swell in an aqueous environment, which is an important parameter for assessing mucoadhesion and drug release behavior. A pre-weighed amount of dried microspheres (e.g., 50 mg) was immersed in a specific volume of swelling medium, typically phosphate buffer (pH 6.8), at room temperature. The microspheres were allowed to swell for a predetermined time interval (e.g., 2 hours) while ensuring they remained undisturbed during the process.

After the specified time, the swollen microspheres were carefully removed from the medium using a fine mesh or filter paper to remove excess surface liquid without causing damage to the microspheres. The swollen microspheres were then weighed immediately. The swelling index was calculated using the formula [22].

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

Where Wo denotes the dry microspheres' initial weight and We denotes the bigger, swollen microspheres' weight in the medium at equilibrium. The experiment was conducted in triplicate for each formulation to ensure accuracy and reproducibility. The swelling index provided insights into the hydrophilic properties and water-absorbing capacity of the microspheres, which directly influence their mucoadhesive characteristics and drug release behaviour.

Mucoadhesion study

The mucoadhesion study was conducted to assess the adhesive properties of the microspheres, which are critical for ensuring prolonged retention at the site of application and improving drug delivery efficiency. The study was performed using an in vitro wash-off test method. Freshly excised mucosal tissues, such as goat intestinal mucosa, were collected and thoroughly washed with phosphate buffer (pH 6.8) to remove any debris. The cleaned tissue was then mounted on a glass slide using adhesive tape to provide a stable surface for the test. A fixed amount of microspheres (e.g., 50 mg) was evenly spread on the mucosal surface. The prepared slide was then attached to the arm of a USP disintegration apparatus and subjected to vertical up-and-down motion in a beaker containing phosphate buffer (pH 6.8) maintained at 37 ± 1°C. The test was conducted at a gentle agitation speed to simulate physiological conditions. At regular time intervals, the number of microspheres still adhering to the mucosal surface was counted. The percentage of adhered microspheres was calculated at each time point to evaluate the mucoadhesive strength. The test was continued until all the microspheres were washed off. The study was repeated in triplicate for each formulation to ensure accuracy and reproducibility. The results of the mucoadhesion study were analyzed to compare the adhesive performance of different formulations. High mucoadhesion percentages indicated better retention and suitability for sustained drug delivery applications [20].

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

In vitro drug release study

The in vitro drug release study was conducted to evaluate the drug release profile from the formulated microspheres under conditions simulating the gastric environment. A USP paddle apparatus was employed as the dissolution equipment, utilizing 900 mL of 0.1N HCl as the dissolution medium. The experiment was carried out at a controlled temperature of 37 ± 0.5°C with a paddle rotation speed of 100 RPM to replicate the physiological conditions of the stomach. At predefined time intervals, 5 mL aliquots of the dissolution medium were withdrawn for analysis, ensuring accurate and consistent sampling. To maintain sink conditions, each aliquot was promptly replaced with an equal volume of fresh dissolution medium. The collected samples were analyzed using a UV spectrophotometer at a wavelength of 280 nm, corresponding to the drug's absorption maximum, to determine the drug concentration in the medium. The cumulative percentage of drug release was calculated for each time point, providing a detailed understanding of the release behavior. This systematic approach allowed for the precise evaluation of the microspheres' sustained release properties. The drug release profiles were further analyzed to elucidate the underlying release mechanism, such as diffusion-controlled or erosion-based release. These insights were instrumental in optimizing the formulation for enhanced therapeutic performance under gastric conditions. The study's robust design ensured the accuracy, reliability, and reproducibility of the results [22].

Statistical analysis

The statistical analysis was performed to validate the experimental findings and assess the significance of differences among the data sets. All experiments were conducted in triplicate,

and the results were expressed as mean ± standard deviation (SD). The data were analyzed using appropriate statistical tools to ensure reliability and reproducibility. To compare the performance of different formulations, a one-way analysis of variance (ANOVA) was employed. This method allowed for the evaluation of statistical differences among multiple groups, such as variations in drug release, encapsulation efficiency, or swelling index across different formulations. When significant differences were observed, post hoc tests, such as Tukey's test, were applied to identify specific groups with statistically significant variations. For analyzing time-dependent parameters, such as drug release profiles, regression analysis was performed to fit the data into various kinetic models (e.g., zero-order, first-order, Higuchi, and Korsmeyer-Peppas equations). The coefficient of determination (r^2) and other statistical metrics were calculated to determine the best-fitting model, providing insights into the release mechanism. Statistical significance was set at a p-value of <0.05 for all analyses. Data processing and statistical evaluations were conducted using software tools such as GraphPad Prism, SPSS, or Microsoft Excel. This rigorous approach ensured the reliability of the results and provided a robust basis for interpreting the study's outcomes.

RESULTS AND DISCUSSION

Particle sizes, Elongation ratio, Uniformity index and microspheres shape

The analyzed formulations (MFC1 to MFC4 and MFH1 to MFH4) exhibit notable differences in particle size, uniformity index (UI), entrapment efficiency ratio (ER), and particle shape, which are critical parameters influencing their performance. Particle size ranged from 5.58 µm (MFH1) to 9.84 µm (MFH4), with MFC formulations generally having smaller particle sizes compared to MFH formulations. Among the formulations, MFC3 displayed the smallest size within the MFC group, while MFH1 was the smallest in the MFH group. Such variations in size are crucial as they impact drug distribution uniformity, release profiles, and formulation stability. The UI values ranged from 0.836 (MFC4) to 1.886 (MFH1), where a lower UI indicates better uniformity. MFC4, with the lowest UI, demonstrated superior uniformity, while MFH1 had the highest UI, suggesting greater size variability.

The entrapment efficiency ratio (ER) varied from 1.177 (MFH4) to 1.369 (MFC1), indicating differences in the capacity to retain the active ingredient. MFC1 exhibited the highest ER, reflecting superior drug entrapment, which is advantageous for therapeutic efficacy. In contrast, MFH formulations generally had slightly lower ER values, possibly due to differences in their composition or preparation techniques. The particle shapes included spherical and non-spherical forms. Most MFC formulations were spherical, except for MFC2, which was non-spherical. Spherical particles are preferred for their superior flow properties and predictable drug release kinetics. Among MFH formulations, MFH1 and MFH2 were spherical, while MFH3 and MFH4 were non-spherical, which could affect packing density and drug release behaviour.

Overall, spherical particles with lower UI values and higher ER, such as MFC1 and MFC3, are likely to offer better performance in drug delivery systems due to their uniformity and efficient drug entrapment. MFC formulations demonstrated a consistent advantage over MFH formulations in these parameters, making them more suitable for applications requiring precise

and predictable performance. Non-spherical formulations, though potentially less uniform, might have niche applications depending on the specific requirements of the drug delivery system. These findings underscore the importance of optimizing particle size, shape, and entrapment efficiency during formulation development to achieve desired therapeutic outcomes.

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

Formulation	**Particle size (µm) ± SD**	**UI**	**ER**	**Shape**
MFC1	9.03 ± 0.16	1.302	1.369 ± 0.12	Spherical
MFC2	8.08 ± 0.15	1.210	1.229 ± 0.14	Non spherical
MFC3	7.07 ± 0.18	1.3188	1.317 ± 0.17	Spherical
MFC4	8.87 ± 0.14	0.836	1.257 ± 0.19	Spherical
MFH1	5.58 ± 0.16	1.886	1.346 ± 0.15	Spherical
MFH2	8.73 ± 0.17	1.117	1.223 ± 0.08	Spherical
MFH3	6.86 ± 0.19	0.889	1.226 ± 0.11	Non spherical
MFH4	9.84 ± 0.19	0.8977	1.177 ± 0.10	Non Spherical

Where UI= Uniformity index and ER= Elongation ratio

Preparation of microspheres: Percentage yield

The data presented in Table 2 provides the percentage yield of various formulations (MFC1 to MFC4 and MFH1 to MFH4), which is an important metric in evaluating the efficiency of the formulation process. The percentage yield ranged from 93.84% (MFH4) to 97.47% (MFC2), indicating good efficiency across all formulations, with minimal material loss during production.

The MFC group displayed consistently higher percentage yields compared to the MFH group. Among the MFC formulations, MFC2 achieved the highest yield (97.47%), reflecting superior process efficiency, while MFC3 showed the lowest yield (94.45%). In contrast, the MFH group demonstrated slightly lower yields overall, with MFH1 and MFH2 achieving relatively better performance (95.59% and 95.78%, respectively), whereas MFH3 and MFH4 exhibited the lowest yields within the group (93.89% and 93.84%).

The differences in percentage yield between the MFC and MFH formulations could be attributed to variations in the composition or processing methods used. Higher yields in the MFC formulations suggest that their preparation techniques were more optimized, minimizing material wastage. In contrast, the MFH formulations, particularly MFH3 and MFH4, may have encountered challenges such as material loss during handling or inefficiencies in the manufacturing process.

Overall, the percentage yield values for all formulations indicate efficient production processes, with yields exceeding 93% in all cases. This suggests that the methods employed for formulation development were well-controlled and reproducible. The higher yields in MFC formulations highlight their potential for scalable production with minimal losses, making them favorable for practical applications. Optimization of the MFH formulations could help achieve comparable yields, further enhancing their production viability.

Table 2. Percentage yield.

Formulation	Percentage yield
MFC1	96.98 ± 0.99
MFC2	97.47 ± 1.01
MFC3	94.45 ± 1.09
MFC4	94.79 ± 1.11
MFH1	95.59± 1.05
MFH2	95.78 ± 1.22
MFH3	93.89 ± 1.18
MFH4	93.84 ± 1.10

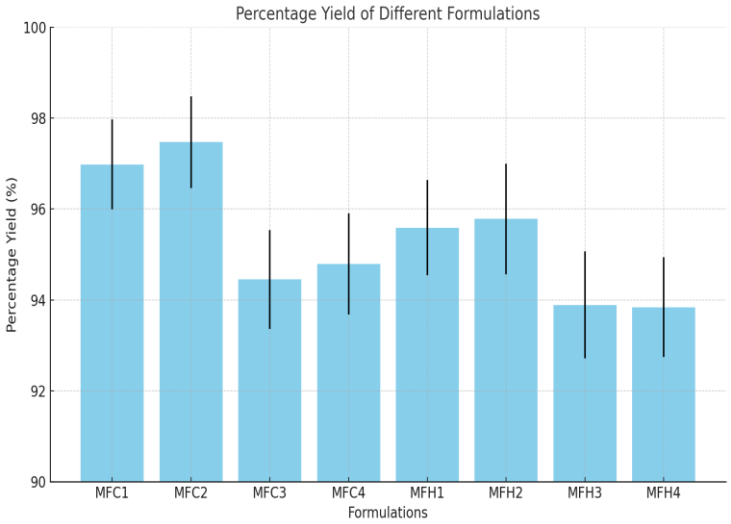

Figure 1. Percentage yield

Encapsulation efficiency, and Drug loading of microspheres

The data in Table 3 highlights the encapsulation efficiency (EE) and loading capacity (LC) of various formulations, which are critical for evaluating their performance in drug delivery. The encapsulation efficiency ranged from 94.86% (MFH3) to 98.46% (MFC2), demonstrating excellent encapsulation across all formulations. MFC formulations generally exhibited slightly higher EE compared to MFH formulations, with MFC2 achieving the highest EE (98.46%), making it the most efficient formulation for incorporating the active compound. In contrast, MFH formulations, while slightly lower in EE, also performed well, with MFH1 showing the highest EE (96.83%) within its group. The variations in EE likely result from differences in the materials or preparation methods used, with higher EE suggesting more favorable interactions between the encapsulating material and the active ingredient.

The loading capacity ranged from 29.87% (MFH3) to 58.56% (MFH1), indicating variability in the formulations' ability to carry the active ingredient relative to the total formulation weight. MFH1 stood out with the highest LC (58.56%), making it highly efficient for delivering larger amounts of the active compound. In the MFC group, MFC1 and MFC2 exhibited high LC values (53.77% and 53.69%, respectively), while MFC3 and MFC4 showed significantly lower

LC (36.77% and 37.91%, respectively). The relatively lower LC in some formulations, such as MFH3 and MFC3, suggests limitations in the capacity of the encapsulating material, which could be addressed through optimization.

Overall, MFC2 emerged as the best-performing formulation in terms of encapsulation efficiency and maintained a high loading capacity, making it an optimal choice for efficient drug encapsulation. Similarly, MFH1 excelled with the highest loading capacity and a high EE, indicating its potential for applications requiring larger payloads. These findings emphasize the need to balance encapsulation efficiency and loading capacity during formulation development, as each parameter significantly impacts the overall effectiveness and suitability of the formulation for specific drug delivery systems.

Table 3. Encapsulation efficiency and Loading capacity

Formulation	EE	LC
MFC1	97.56	53.77
MFC2	98.46	53.69
MFC3	95.39	36.77
MFC4	95.77	37.91
MFH1	96.83	58.56
MFH2	96.78	52.79
MFH3	94.86	29.87
MFH4	94.91	35.86

Where EE= Encapsulation efficiency, LC= Loading capacity

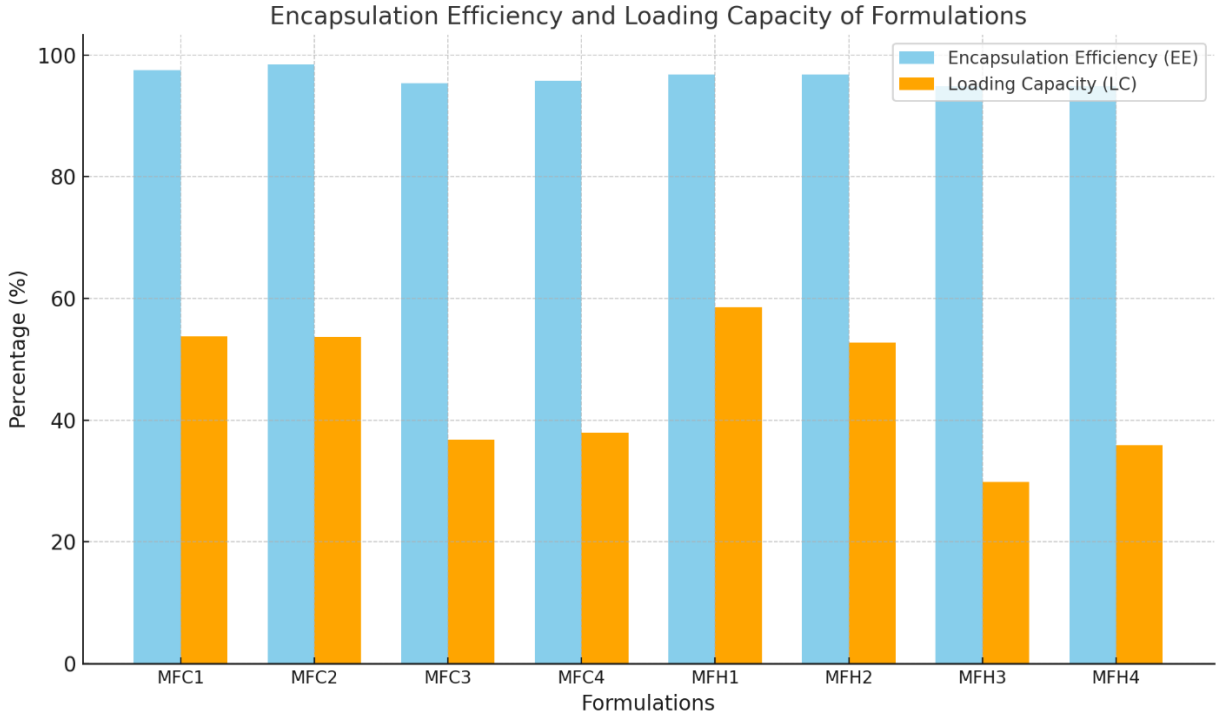

Figure 2. Encapsulation efficiency and Loading capacity

Scanning Electron Microscopy (SEM)

The scanning electron microscopy (SEM) analysis revealed significant insights into the surface morphology of the formulations. The micrographs indicated that the majority of the particles exhibited a smooth and uniform surface, with spherical morphology observed predominantly in formulations such as MFC1, MFC3, and MFH1. Non-spherical shapes with slightly irregular surfaces were noted in formulations MFC2, MFH3, and MFH4. The particle size observed in the SEM images was consistent with the size measured through particle size analysis, confirming the reliability of the preparation method. The uniformity in the spherical particles suggests enhanced flow properties and potential for predictable drug release, while the non-spherical particles may affect packing density and drug release kinetics. The SEM images also highlighted the absence of significant aggregation, indicating efficient dispersion of the particles during formulation. These morphological characteristics underscore the importance of particle shape and surface uniformity in achieving optimal performance in drug delivery systems.

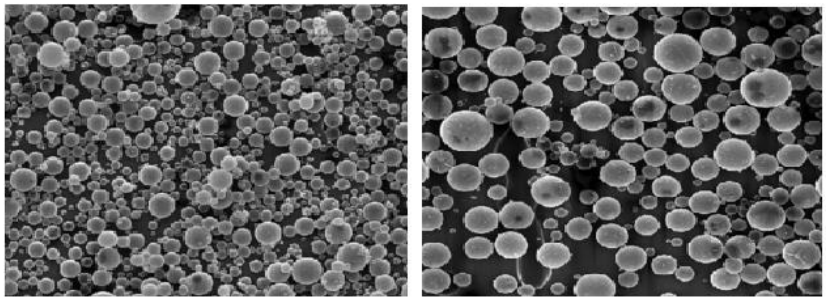

Figure 3. SEM photomicrograph of the formulation of the microsphere (MFC)

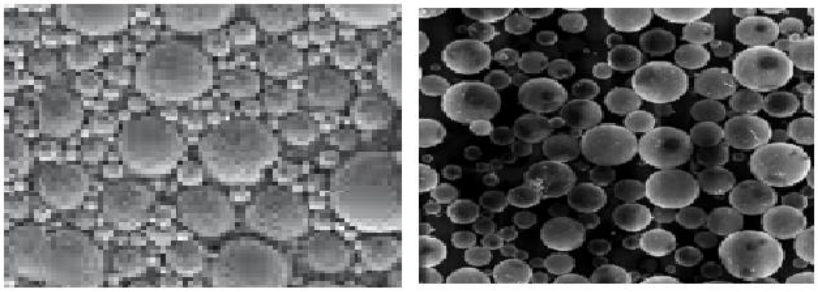

Figure 4. SEM photomicrograph of the formulation of the microsphere (MFH)

Swelling Index

The swelling index varied significantly across the formulations, ranging from 0.22 (MFC4) to 0.98 (MFC1 and MFH1). Formulations such as MFC1, MFC3, and MFH1 exhibited the highest swelling indices (0.97–0.98), indicating their superior ability to absorb fluid and expand upon hydration. In contrast, formulations like MFC2, MFC4, MFH2, and MFH4 demonstrated lower swelling indices (0.22–0.28), reflecting limited hydration capacity. These variations suggest differences in the hydrophilic nature and polymeric structure of the formulations. A higher swelling index is generally beneficial for sustained drug retention and controlled release; however, excessive swelling may lead to rapid erosion or compromised mucoadhesion. Thus, formulations with moderate swelling indices may achieve an optimal balance between hydration and structural integrity.

Mucoadhesioon

The mucoadhesion percentage showed considerable variability, ranging from 52.38% (MFH4) to 78.56% (MFC2). MFC2 emerged as the top-performing formulation with the highest mucoadhesion, suggesting strong adhesive interactions that are critical for prolonged retention and improved drug absorption. On the other hand, MFH4 demonstrated the weakest mucoadhesion (52.38%), indicating reduced interaction with the mucosal surface. Interestingly, formulations with lower swelling indices, such as MFC2, displayed superior mucoadhesion, likely due to a dense polymer structure enabling robust adhesive forces. In contrast, formulations with higher swelling indices, such as MFC1 and MFH1, exhibited moderate mucoadhesion, indicating that excessive swelling might dilute adhesive interactions. These findings underscore that mucoadhesion depends more on the polymer's adhesive properties than its swelling behavior, emphasizing the need for a balanced design to achieve optimal performance in mucoadhesive systems.

Table 4. Index of swelling and Mucoadhesion percentage

Formulation	**Swelling index**	**Percentage Mucoadhesion after 600 min**
MFC1	0.98 ± 0.027	66.72 ± 1.43
MFC2	0.27 ± 0.022	78.56 ± 1.66
MFC3	0.97 ± 0.031	72.66± 1.51
MFC4	0.22 ± 0.030	59.28 ± 1.43
MFH1	0.98 ± 0.022	69.28 ± 2.01
MFH2	0.27 ± 0.023	57.33± 1.42
MFH3	0.97 ± 0.028	63.47 ± 1.25
MFH4	0.28 ± 0.029	52.38 ± 1.22

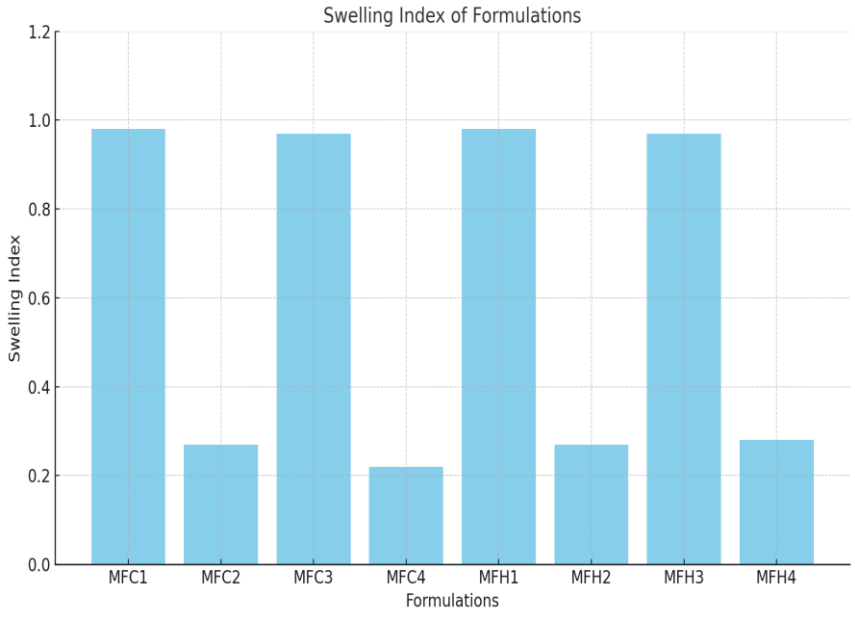

Figure 5. Swelling Index of Formulations

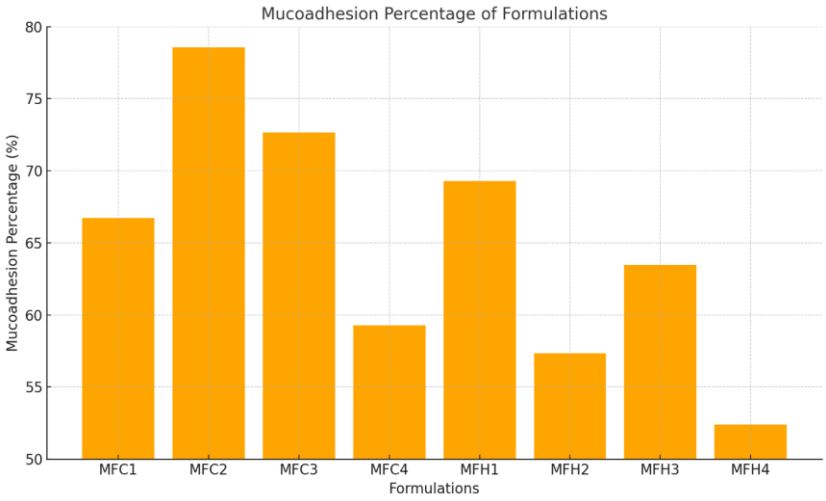

Figure 6. Mucoadhesion percentage after 600 minutes

In vitro drug release study

The data in Table 5 outlines the in vitro drug release profiles of various formulations (MFC1 to MFC4 and MFH1 to MFH4) at pH 1.2 over 12 hours. The results reveal distinct patterns in drug release behavior, indicating differences in formulation composition, structure, or drug-polymer interactions that influence release kinetics. Formulations MFC1 and MFC2 exhibited the highest cumulative drug release at the end of 12 hours, with values of 100.25% and 98.25%, respectively. These formulations demonstrated consistent and controlled release rates throughout the study, maintaining steady increases over time. MFC1's slightly higher drug release indicates superior formulation efficiency, likely due to optimized polymer-drug interactions facilitating prolonged and complete drug diffusion. Among the MFC group, MFC3 and MFC4 displayed slightly lower release rates, with cumulative releases of 96.34% and 94.35% at 12 hours, respectively. These formulations exhibited slower release during the early time points, likely influenced by differences in swelling behavior or polymer density, which may have restricted drug diffusion initially. In the MFH group, MFH1 and MFH2 demonstrated high release rates of 93.45% and 92.99% at 12 hours, respectively, indicating effective drug release but slightly lower efficiency compared to MFC formulations. MFH3 and MFH4 showed the lowest release rates among all formulations, reaching only 92.36% and 91.45% at 12 hours. This trend was consistent across all time points, with MFH4 consistently lagging behind the other formulations, potentially due to a denser polymer matrix or reduced swelling capacity limiting drug diffusion. Overall, MFC formulations exhibited superior drug release profiles compared to MFH formulations, suggesting that the MFC group is better suited for sustained release applications. MFC1, in particular, demonstrated the most efficient and complete drug release, making it an optimal choice for controlled drug delivery at acidic pH. These findings highlight the importance of optimizing formulation parameters, such as polymer composition and structure, to achieve desired release profiles tailored to specific therapeutic needs.

Table 5. Drug release in vitro at pH 1.2

Formulation	MFC1	MFC2	MFC3	MFC4	MFH1	MFH2	MFH3	MFH4
1hr	20.88±1.23	19.11±1.16	18.1±1.19	17.74±1.03	19.09±1.23	17.58±1.23	16.45±1.23	13.13±1.12
2hr	32.49±1.26	30.89±1.24	29.76±1.28	27.99±1.37	29.77±1.33	28.22±1.26	27.21±1.24	22.94±1.24
3hr	37.22±1.36	35.21±.26	33.44±1.46	33.1±1.26	34.44±1.28	34.22±1.26	32.89±1.28	31.0±1.3
4hr	40.22±1.46	39.74±1.26	39.21±1.26	37.89±1.46	37.44±1.46	37.22±1.41	35.11±1.46	33.21±1.46
5hr	49.64±1.59	48.21±1.41	42.62±1.46	41.22±1.46	47.26±1.41	42.69±1.46	40.72±1.26	39.91±1.59
6hr	58.21±1.41	57.19±1.46	55.09±1.41	50.87±1.26	52.98±1.46	52.25±1.41	51.22±1.46	43.22±1.46
7hr	63.2±1.46	62.54±1.41	62.09±1.46	60.9±1.46	59.21±1.41	58.09±1.46	56.64±1.59	53.2±1.39
8hr	67.78±1.59	67.34±1.59	67.22±1.41	64.44±1.41	65.16±1.46	62.53±1.59	62.2±1.46	61.8±1.46
9hr	73.22±1.41	71.22±1.46	70.34±1.46	69.78±1.46	70.64±1.46	68.89±1.46	67.22±1.41	62.56±1.59
10hr	80.89±1.46	79.88±1.41	76.69±1.59	76.02±1.59	79.11±1.59	77.22±1.59	75.13±1.46	72.44±1.46
11hr	89.25±1.41	87.26±1.59	85.81±1.46	85.35±1.46	87.44±1.46	84.44±1.46	83.16±1.59	82.1±1.59
12hr	100.25±1.46	98.25±1.46	96.34±1.41	94.35±1.59	93.45±1.59	92.99±1.41	92.36±1.46	91.45±1.46

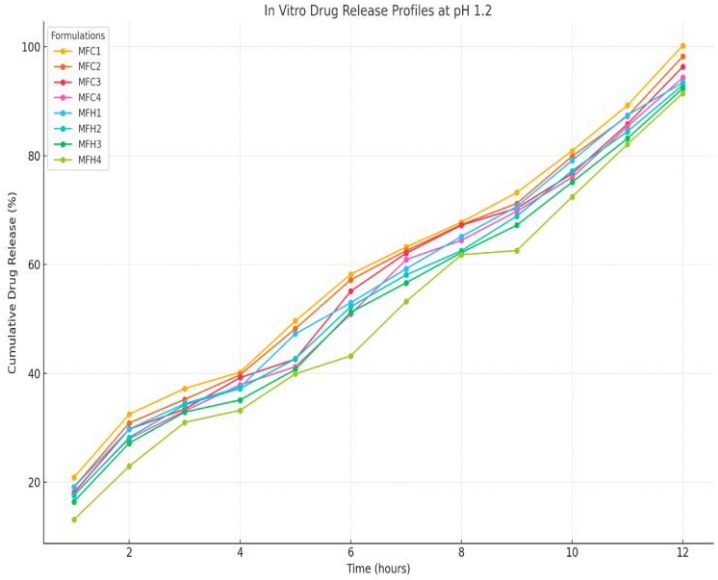

Figure 7. Drug release in vitro at pH 1.2

The optimized formulation and the method

The study demonstrated that the chemical stabilization method was the superior approach for fabricating DGL-loaded mucoadhesive microspheres, as evidenced by the consistent performance of the MFC formulations. This method allowed for efficient drug encapsulation, with MFC1 achieving the highest encapsulation efficiency (97.56%) and a well-balanced loading capacity (53.77%). The chemical stabilization process, facilitated by glutaraldehyde as a cross-linking agent, resulted in microspheres with uniform particle size, spherical morphology, and enhanced structural stability, as confirmed by scanning electron microscopy. Among all formulations, MFC1 emerged as the optimized formulation, exhibiting a favorable swelling index (0.98) and high mucoadhesion (66.72%), ensuring prolonged retention at the mucosal surface. Its sustained drug release profile, achieving 100.25% cumulative release over 12 hours at pH 1.2, highlighted its capability for gastric-targeted applications. These results underscore the critical role of formulation techniques and polymer ratios in optimizing the performance of microspheres. The chemical stabilization method, combined with a 1:1 drug-to-polymer ratio in MFC1, proved to be the most effective strategy for achieving the desired balance of encapsulation efficiency, mucoadhesion, and drug release properties, making it a promising candidate for sustained-release drug delivery systems.

CONCLUSION

The study successfully developed and characterized DGL-loaded mucoadhesive microspheres using two stabilization techniques. The chemical stabilization method proved to be the most effective, yielding formulations with superior encapsulation efficiency, structural stability, and consistent performance. Among all formulations, MFC1 demonstrated the highest optimization, achieving an ideal balance of encapsulation efficiency (97.56%), swelling behavior (0.98), and mucoadhesion (66.72%). Its sustained drug release profile, achieving complete drug release over 12 hours in a simulated gastric environment, underscores its suitability for prolonged gastric retention and targeted drug delivery. The heat stabilization method, while producing stable formulations, showed comparatively lower encapsulation efficiencies and slower release profiles. The findings highlight the critical influence of formulation techniques and polymer ratios on microsphere performance, emphasizing the chemical stabilization method's reliability for developing advanced drug delivery systems. MFC1 stands out as a promising candidate for gastric-targeted therapeutic applications, paving the way for future studies focusing on in vivo efficacy, scalability, and long-term stability. These insights contribute to the advancement of sustained-release mucoadhesive drug delivery technologies, offering improved therapeutic outcomes and patient compliance.

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