Original Article

Formulation and Evaluation of Sodium Alginate and Guar Gum Based Glycyrrhizin Loaded Mucoadhesive Microspheres for Management of Peptic Ulcer

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ABSTRACT

Background: Glycyrrhizin (GZ) is a bioactive ingredient of *Glycyrrhiza glabra*, reported for various therapeutic effects including gastro-protection. It has been associated with low absorption, early elimination, short half-life and poor bioavailability. **Objectives:** Aim of the current study was to formulate GZ loaded mucoadhesive microspheres by using mucopolymers like sodium alginate and guar gum for the management of peptic ulcer. **Methods:** Various GZ loaded microspheres (GZ-MS1-3) were prepared by an emulsification-crosslinking technique. These formulations were developed with different proportions of guar gum and sodium alginate. The formulations were characterized and evaluated by various parameters including particle size, zeta potential, entrapment efficiency (% EE), % yield, SEM, FTIR, swelling index, mucoadhesive efficiency, *in vitro* drug release and *in vivo* antioxidant activities. **Results:** Result stated that suitable particle size (50.18 ± 1.15 μm), zeta potential (-31.12 ± 2.16 mV), %EE (92.67 ± 1.91) and % yield (97.45 ± 1.83) was achieved with optimized formulation, GZ-MS1. Significant (***$P<0.001$) swelling index (0.94 ± 0.04) and mucoadhesive efficiency (95.98 ± 3.62%) was obtained with GZ-MS1. GZ-MS1 showed maximum drug release profile (94.57 ± 4.03 %) in simulated gastric fluid (SGF, pH 1.2) at 37 ± 0.5°C for 24 h. FTIR study confirmed that there was no interaction observed between GZ and excipients. **Conclusion:** Sustained release profile of the optimized formulation was achieved due to significant mucoadhesive efficiency of the sodium alginate and guar gum. Thus, the mucoadhesive microspheres of GZ would be an effective strategy for the management of peptic ulcer.

Key words: Mucoadhesive microspheres, Glycyrrhizin, Mucopolymers, Sodium alginate, Guar gum, Sustained release, Peptic ulcer.

INTRODUCTION

Peptic Ulcer (PU) is a multifactorial gastrointestinal tract disease, concerned with open sores or lesions in the mucosal lining of the stomach and duodenum. Most of the people have suffered with PU globally. PU has been connected with indigestion, nausea, heartburn, dyspepsia, bleeding and epigastric pain.[1,2] *Helicobacter pylori* infection and use of nonsteroidal anti-inflammatory drugs (NSAIDs) are the main factors for causing PU.[3,4] The inflammatory cytokines like IL-17A, IL-8 IL-1β and TNF-α are responsible for the pathogenesis of PU in humans and considered as major factors for prevalence of PU in developing countries.[5,6] There are numerous classes of modern therapy available for the management of PU which include antibiotics, antacids, anticholinergics, antisecretory agents (proton pump H^+/K^+ ATPase inhibitors), cytoprotective agents and antihistaminics (H_2 receptor antagonist).[7] But their therapeutic uses are limited due to side effects, the incidence of relapses and multi-drug resistance. Thus, herbal medicine can be used as a safe and alternative drug for the treatment of PU. A wide array of botanicals

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and their bioactive phytoconstituents have potential to treat several kinds of diseases like PU.[3] GZ is a potent bioactive of *Glycyrrhiza glabra,* reported to be antiulcer, antiinflammatory, antioxidant, antibacterial, anticancer, hepatoprotective and antiviral. GZ is a BCS-II molecule and its therapeutic activities are limited due to poor absorption (log p 2.8), lowest bioavailability (~1%) and low half-life (3.5 h).[8-11] GZ possessed its potent anti-inflammatory activity through selective inhibition of prostaglandin E2, cyclooxygenase, lipoxygenase and phospholipase A2.[12]

The novel oral mucoadhesive drug delivery system has the ability of retaining in the stomach for longer times and can release the drug content slowly so that an effective level of drug can be provided to its site of absorption (stomach) to heal the ulcer. Moreover, these systems can channel the local drug action in the upper region of the small intestine, which can be suitable for the treatment of duodenal ulcer as well (Figure 1). It is utmost important in drug delivery system to meet the current demand of drug therapy by maintaining drug concentration in blood circulation for prolonged times.[13-15]

Mucoadhesive microspheres are tiny spherical units (~1000 μm), have the ability to form bioadhesion to the gastric mucosa which restricts gastric emptying of formulation through the pyloric sphincter. These carrier systems can be spread out homogeneously over the entire region of the stomach and upper small intestine, which can facilitate improved absorption and localized action of drug.[16,17] The bioadhesion of these carriers are generally facilitated by muco-polymers, having the ability to adhere to the surface of epithelial tissues of the stomach by intimate contact. This results in delaying gastric emptying time, thus the time of retention of the product in the gastric region is enhanced.[18]

Sodium alginate (SA) is a polysaccharide type polymer (anionic in nature), used as a mucoadhesive and gelling agent in the development of microcarriers. It has two units, β-1,4-$_D$-manurunic acid and α-1,4-$_L$-guluronic acid in their structure that offers optimum mucoadhesive properties including pH-sensitivity, cross-linking capability, biocompatibility and biodegradability. These features are more suitable for gastric delivery.[19,20] Guar gum (GG) is another plant derived mucopolymer having mannose (1→4)-β-$_D$-mannopyranosyl and galactose α-$_D$-galactopyranosyl structural unit, joined through (1→6) bond. It is having amazing gelling, mucoadhesive and biodegradable property.[21,22] Thus, the current study was aimed to prepare SA-GG based glycyrrhizin-mucoadhesive microspheres for gastric delivery.

MATERIALS AND METHODS

Materials

The pure GZ (assay 99%) was purchased from Sigma Chemicals, Germany. SA and GG were purchased from Finar Chemicals Ltd., Ahmedabad, India. Castor oil and Span 80 was obtained from Merck, Mumbai, India. Analytical grade other chemicals, reagents and deionised water were used in the experiment.

Preparation of Standard Solution of Glycyrrhizin

SGF (pH 1.2) was prepared by incorporation of HCl (7 mL), NaCl (2 g) and pepsin (3.2 g) in distilled water (1000 mL). The accurate amount of GZ (10 mg) was transferred into a volumetric flask (100 mL), then the volume was adjusted up to the mark by SGF to get a final concentration of 100 μg/mL. It was considered as a stock to prepare aliquots further.

Preparation of Different Aliquots of Glycyrrhizin

UV-spectrophotometer, Shimadzu 1700, Japan was used for analysis of the sample. From the standard stock solution, different aliquots 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2 and 2.4 mL were taken and diluted to get concentration of 2-24 μg/mL respectively. These solutions were passed through the syringe filter and absorbance of each sample was recorded at 258 nm by UV-spectrophotometer.

Preparation of Glycyrrhizin Loaded Mucoadhesive Microspheres

GZ unloaded and loaded mucoadhesive microspheres (GZ-MS) were fabricated by modified emulsification-crosslinking method (Figure 2).[21] Briefly, GZ (200 mg) was dissolved in 100 g deionised water containing tween 80 (1%, w/w). A polymer dispersion system composed

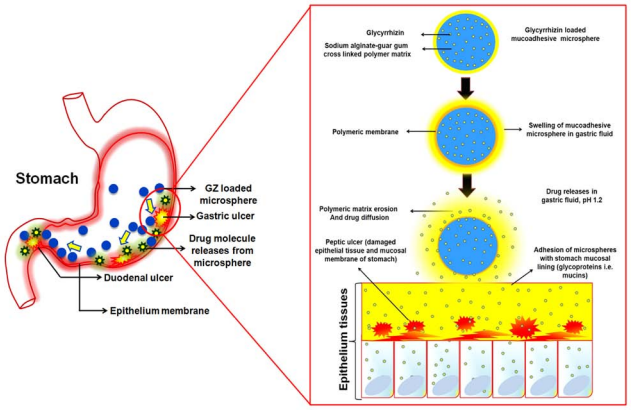

Figure 1: Schematic representation of peptic ulcer (gastric ulcer and duodenal ulcer) and glycyrrhizin loaded mucoadhesive microsphere based treatment approach for the peptic ulcer

of sodium alginate (1.5-2.5%, w/w) and guar gum (500, 600 and 700 mg) in distilled water (100 g), was utilized for preparation of GZ-MS. To get a homogeneous mass, these dispersions were allowed to swell completely at room temperature for 2 h and mixed by digital magnetic stirrer (Remi, India). The ratio between GZ and polymers was 1:10, 1:13 and 1:16 w/w (Table 1). Then polymer dispersion was added dropwise into the dispersion medium (castor oil containing span 80, concentrated sulfuric acid and glutaraldehyde) by syringe-needle (24 G size). It was stirred at a constant speed of 3000 rpm for 4 h at 50°C to produce microspheres. The formed microspheres were filtered and washed with isopropyl alcohol. Sodium bisulfite was used to remove the glutaraldehyde residue in the formulations. The microspheres were dried at 50°C under a hot air oven and kept for 24 h in vacuum desiccators till further studies.

Characterization and Evaluation of Mucoadhesive Microspheres of Glycyrrhizin

Particle Size, Polydispersity Index (PDI) and Zeta Potential Analysis

The average particle size, PDI and zeta potential of suspended mucoadhesive microspheres (GZ-MS1-3, 10 mg/mL) were analyzed by Zetasizer (Nano ZS90, Malvern instruments Ltd., UK) with a 50 mV laser. Analysis was performed at room temperature (25 ± 0.5°C).

Percentage Yield and Entrapment Efficiency (%EE)

%Yield and %EE of the GZ loaded mucoadhesive microspheres (GZ-MS1-3) were determined as per our previous reported method.[23] Sample was analyzed through UV-spectrophotometer (Shimadzu 1700, Japan) at 258 nm.

Percentage (%) yields of different formulations, GZ-MS1-3 were calculated by the following formula.

% Yield = (Weight of microspheres/ Weight of all non-volatile components) × 100..... (1)

%EE of the glycyrrhizin loaded mucoadhesive microspheres (GZ-MS1-3) were determined as per the reported method.[23] Briefly, specific weight (100 mg) of microspheres was crushed in a glass mortar to make powder. Then it was transferred into a volumetric flask containing SGF (100 mL) and ultrasonicated to extract out the drug content in the medium. Thereafter, the suspension was filtered through membrane filters (0.45 µm) and analyzed through a UV-spectrophotometer (Shimadzu 1700, Japan) at 258 nm. Each determination was made in triplicate (n = 3). The %EE was estimated based on the following formula.

% EE = (Actual drug content/Theoretical drug content) × 100..... (2)

Degree of Swelling

The swelling degree of GZ-MS1-3 was performed in SGF medium (pH 1.2) to ensure their swelling ability.[23] *USP type 1 (basket type) dissolution test apparatus* was utilized to carry out the swelling degree of the formulations in SGF at 37 ± 0.1°C. An appropriate amount of sample (100 mg) was transferred into the basket and allowed to swell for 12 h. Then *the wet* microspheres *were taken out and treated with blotting paper to remove* excess SGF drops from the surface of the microspheres. The weight of the wet microspheres was recorded and their swelling property was calculated from the following formula.

Degree of swelling (α) = $\omega_s - \omega_o / \omega_o$ ..... (3)

Where, ω_o = weight of microspheres before swelling and ω_s = weight of microspheres after swelling.

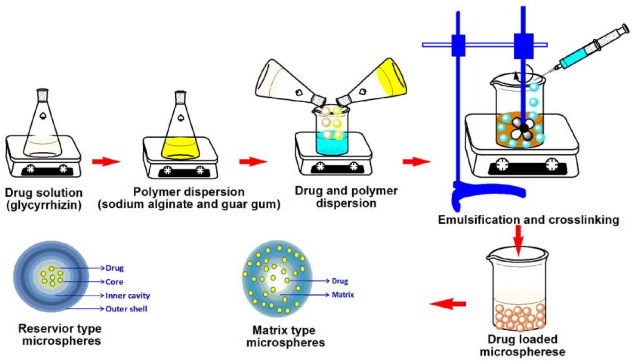

Figure 2: Schematics of preparation of glycyrrhizin loaded mucoadhesive microspheres.

Table 1: Formulation of glycyrrhizin loaded different mucoadhesive microspheres.

Formulation	Drug : Polymer ratio	Glycyrrhizin (mg, w/w)	Polymers	
			Guar gum (mg, w/w)	Sodium alginate (mg, w/w)
GZ-MS1	1:10	200	500	1500
GZ-MS2	1:13	200	600	2000
GZ-MS3	1:16	200	700	2500

In vitro Mucoadhesiveness of Glycyrrhizin Loaded Formulations

The mucoadhesive properties of GZ-MS1-3 were studied through modified *in vitro* wash-off technique.[24] Briefly, freshly excised pieces of goat stomach (collected from a local slaughter house) were fixed on a glass slide (7.5 × 2.5 cm) with the help of dual adhesive tape. The USP tablet disintegration test apparatus was used to perform this study. Measured number of microspheres were put over the mucosal layer of slides and attached in to a arm of the apparatus. Then, they were placed into a 900 mL beaker (SGF, pH 1.2, 37 ± 0.5°C) and movement (up and down) of the was maintained for 12 h. The % mucoadhesiveness of the GZ-MS1-3 was noted at different time intervals and measured by the following formula.

% Mucoadhesiveness = Quantity of microspheres adhered / Quantity of microspheres spread × 100 (4)

In vitro Drug Release Study

In vitro drug release studies of GZ-MS1-3 were performed in SGF (pH 1.2) by using a single dissolution test (paddle type) apparatus. Different formulations (GZ-MS1-3, 100 mg) were spread gently over the surface of the beaker containing 900 mL SGF buffer medium (37 ± 0.5°C) and it was agitated at 100 rpm for 24 h. The sink condition was maintained throughout the study by withdrawing 5 mL of sample and replacing with the equivalent volume of fresh dissolution medium. Samples were filtered with the help of a 0.45 µm syringe filter and analyzed by spectrophotometer at 258 nm. GZ concentrations in different samples were calculated based on a standard calibration curve. All experiments were performed in triplicate (n = 3).

Spectrophotometric Analysis

Pure GZ (10 mg) was poured into different volumetric flasks. Different excipients like sodium alginate (10 mg) and guar gum (10 mg) were added into flasks and volume was adjusted up to 10 ml with SGF (pH 1.2) then flasks were agitated for 6 h and stored overnight. Samples were filtered, suitably diluted and analyzed at 258 nm by UV-spectrophotometer.

Fourier Transforms Infrared (FTIR)

The FTIR spectrum investigation was carried out to confirm the compatibility of pure glycyrrhizin with different excipients which was used for preparation of optimized mucoadhesive microspheres (GZ-MS1). The KBr discs of individual ingredients, i.e. pure glycyrrhizin, GZ-MS1, sodium alginate and guar gum were prepared and analysed by using a FTIR spectrophotometer (Perkin Elmer, USA) in the range of 4000-500 cm^{-1}.

Surface Electron Microscopy (SEM)

The shape and surface morphology of optimized formulation (GZ-MS1) and placebo GZ-MS1 was studied by using SEM (Jeol JSM-1600, Tokyo, Japan). Briefly, aluminum stub and double-sided adhesive carbon tape were used for preparation of samples. Formulations were sprinkled over the surface of the tape containing stub for sampling. Sample coating material, platinum was applied to make a fine layer (300 °A thickness) onto the specimen-stub by using sputter-coater under argon atmosphere and high-vacuum condition. These specimens were analysed by SEM and their photomicrographs were recorded at different magnifications.

Drug Release Kinetic Models

Various drug release kinetic models including Zero order, First order, Higuchi's and Korsmeyer-Peppas were subjected for formulations, GZ-MS1-3 to forecast their mechanism of drug release.

Stability Analysis

GZ-MS1 was subjected for stability study at various conditions of 25 ± 2°C / 60 ± 5 % RH, 30 ± 2°C/ 65 ± 5 % RH and 40 ± 2°C / 75 ± 5 % RH according to the guideline of ICH. Briefly, *prior analysis, product (*GZ-MS1*) was* stored *in a tightly closed container (amber colored glass bottles). Then, the product* was *kept* in a chamber of stability testing equipment where modulated environmental conditions were provided for a period of 180 days. The formulations were observed for changes in their morphological behaviour, particle size, zeta potential, physical appearance and drug content at an interval of 45, 90 and 180 days.

Evaluation of Antioxidant Potential of GZ-Loaded Formulations against Ethanol Mediated Gastric Damages

Animals

Male Wistar rats (~200 g) were used in this experiment, animals were stored in 5 groups (n = 6) in different cages and acclimatized at room temperature (25 ± 0.5°C and 45-55% RH) for 1 week with 12 h light/dark cycles. All animals had free access to feed and purified water. The experimental protocol was approved by the Institutional Animal Ethics Committee.

Dosing

Antioxidant potential of GZ and GZ-MS1 were performed as the reported method.[25] Prior to the

experiment, all the rats were fasted for overnight with free access to water. Animals were divided into following different groups like group I: normal control (0.5% tween 80, 5 mL/kg body weight, p.o.), group II: disease control (1 mL/200 g ethanol, p.o.), group III: GZ200 (200 mg/kg GZ, p.o.), group IV: GZ-MS1 (~200 mg/kg GZ, p.o.) and group V: placebo (500 mg/kg, p.o.). An ulcerogenic agent, absolute ethanol (95-99%, 1 mL/200 g) was given orally to all animal groups except the normal group after 1 h. All the rats were sacrificed and stomachs were removed after 1 h of ethanol administration. Glandular gastric tissues were dissected out and washed with ice-cold saline. These gastric tissues were homogenized into several fragments and homogenates were made in phosphate-buffer saline (0.1 M, pH 7.4). These homogenates were centrifuged at 13500 rpm for 5 min using Spinwin MC-02, Tarson, India. Then pure supernatant layers of the homogenates were taken out and kept at -20°C till further uses.

Antioxidant Marker Enzymes Estimation in Rat Gastric Tissue

Estimation of catalase (CAT),[26] thiobarbituric acid reactive substances (TBARS),[27] superoxide dismutase (SOD)[28] and glutathione peroxidase (GPx)[29] levels in rat stomach homogenate were carried out. In the rat gastric homogenate, the total content of protein was determined.[30] Protein content was estimated as units/mg.

Statistical Analysis

One-way ANOVA-Dunnet's *post hoc* test was performed for statistical analysis of various experimental data. The data were processed through Graph Pad Prism, San Diego, CA, USA. Mean ± SD and Mean ± SEM were expressed for all data. The *"P"* value less than 0.05 ($P < 0.05$) was considered to be significant throughout the statistical treatment.

RESULTS

Characterization and Evaluation of Glycyrrhizin Loaded Mucoadhesive Microspheres

Particle Size, PDI and Zeta Potential Analysis

The particle size, PDI and zeta potential of the GZ loaded mucoadhesive microsphere formulations (GZ-MS1-3) were analysed. The detail features of formulations were explained in Table 2. The particle size (Figure 3A), PDI and zeta potential (Figure 3B) were found to be 50.18 ± 1.15 µm, 0.62 ± 0.11 and -31.15 ± 2.16 mV for GZ-MS1 respectively.

%Yield and %Entrapment Efficiency

%Yield of the different formulations, GZ-MS1-3 was shown in Table 2. The maximum yield (97.45 ± 1.83%) of the formulation was obtained with GZ-MS1. It may be due to an appropriate ratio of drug: polymer (1:10 w/w) for development of microspheres at a constant stirring rate (3000 rpm). %EE of the GZ loaded various formulations (GZ-MS1-3) were represented in Table 2. GZ-MS1 showed maximum (92.67 ± 1.91%) entrapment efficiency which may be due to the suitable composition of SA-GG in the microspheres.

Degree of Swelling and *in vitro* Mucoadhesiveness

Swelling properties of different formulations were performed in SGF medium (pH 1.2) and results were represented in Figure 3C. Swelling degrees (*a*) were found to be in the range of 0.94 ± 0.04 to 0.62 ± 0.04 for GZ-MS1-3. The best swelling degree value (0.94 ± 0.04) was achieved with GZ-MS1 for an extended period (12 h), which may be suitable for retaining in the stomach. As a result, it was stated that the degree of swelling was higher for GZ-MS1 due to cross-linked polymers, SA-GG. Cross-linking of polymers with

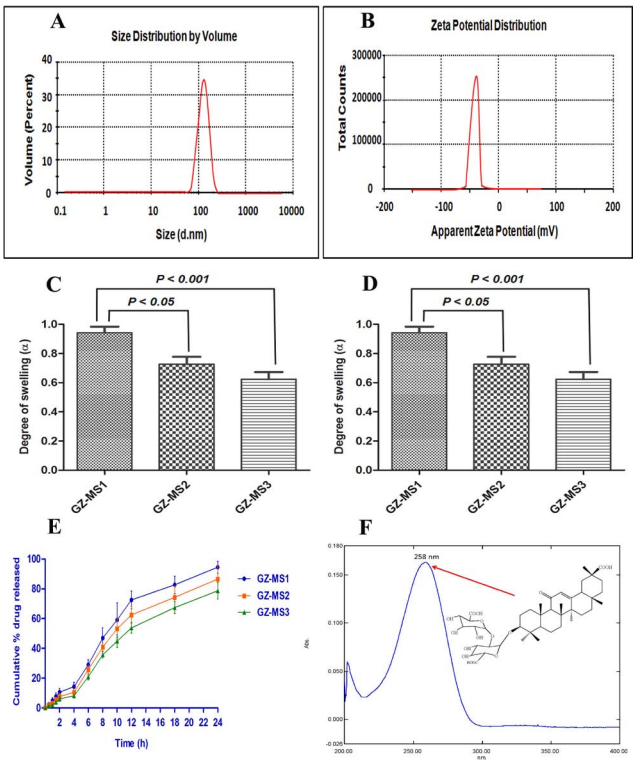

Figure 3: Particle size (A), zeta potential of GZ-MS1 (B), swelling degree (C), mucoadhesive properties (D), *in vitro* drug release profile (E) of glycyrrhizin loaded microspheres (GZ-MS1-3) and UV-spectrum of pure glycyrrhizin in SGF buffer at 258 nm (F). Values were Mean ± SD ($n = 3$). **$P < 0.05$ and ***$P < 0.001$.

glutaraldehyde extended the swelling process in SGF and *in vitro* digestion of the GZ-microspheres.

Mucoadhesive behaviors of various formulations, GZ-MS1-3 were performed to check out the adhesion efficiency of microspheres to the gastric mucosa for prolonged drug release. Results have been shown in Figure 3D. Mucoadhesive characteristics were found to be 95.98 ± 3.62 to 66.28 ± 4.48% for different formulations (GZ-MS1-3). The highest mucoadhesive behaviour (95.98 ± 3.62%) was achieved with GZ-MS1. This may be due to mucoadhesive affinity of the crosslinked SA-GG towards the gastric mucosal membrane containing glycoproteins.

In vitro Drug Release Study

GZ release profiles of different formulations, GZ-MS1-3 were studied in SGF dissolution medium (pH 1.2) at 37°C. Drug release profile was recorded in the range of 94.57 ± 4.03 to 78.73 ± 5.51% for GZ-MS1-3 respectively. The maximum GZ was found to be 94.57 ± 4.03 % with GZ-MS1 for an extended period of 24 h as compared with other formulations. Detailed *in vitro* drug release profiles of different formulations were represented in Figure 3E.

Spectrophotometric Analysis

UV-spectrum analysis of the GZ and along with their formulation excipients were shown in Figure 3F. The wavelength of the GZ-sodium alginate, GZ-guar gum or in combination was the same as pure GZ (258 nm) when assayed spectrophotometrically. This indicates that there were no significant interactions seen between polymers and GZ used in the formulations.

FTIR

FTIR analysis was performed to check the drug-excipient compatibility and detailed FTIR spectrum of GZ, GZ-MS1, SA and GG was shown in Figure 4(A-D). Spectrum of GZ showed prominent -OH stretching band at 3223.05 cm^{-1}, C-H stretching band at 2947.23 cm^{-1}, 2875.86 cm^{-1} and -C=O stretching peak at 1722.43 cm^{-1}, C=C stretching vibration at 1656.85 cm^{-1}, C-H deformation at 1452.04 cm^{-1} and C-OH stretching at 1053.13 cm^{-1}. But in the case of GZ-MS1, these bands were slightly shifted due to entrapment GZ in the polymer matrix. Thus, the FTIR spectrum confirmed the presence of GZ in the formulation and it suggested that the excipients used in the development of the mucoadhesive microspheres were compatible with GZ. No drug-excipient interactions were seen in the formulation.

SEM

Surface structure and morphology of the optimized formulation, GZ-MS1 and placebo were shown in Figure 5(A-D). Photomicrographs of samples demonstrated that the particles were spherical and normal in shape at 400x magnifications. However, rough surface and matrix structure of both the formulations were observed at high magnification (1500x) this could be due to the crosslinking of SA and GG.

Drug Release Kinetics

In vitro release data of GZ-MS1-3 were analysed with various kinetic models like Zero order, First order,

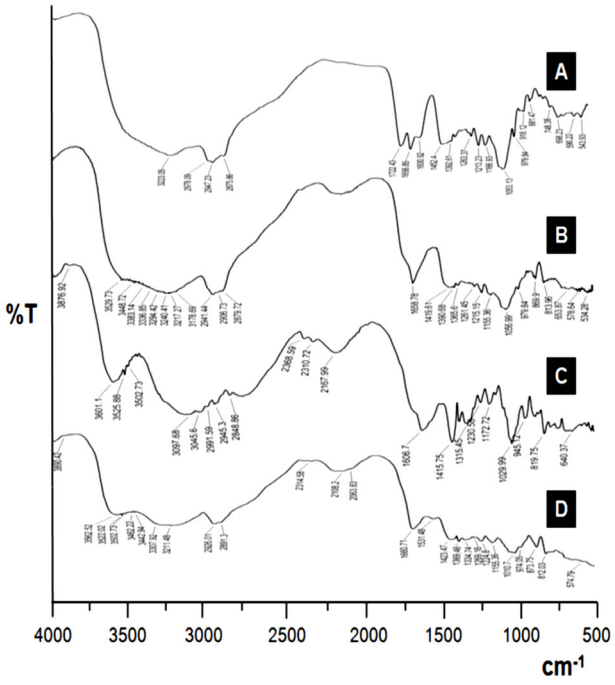

Figure 4: FTIR spectrum of pure glycyrrhizin (A), GZ-MS1 (B), sodium alginate (C) and guar gum (D).

Table 2: Characterization of GZ loaded different mucoadhesive microspheres.

Formulations	Particle size (µm)	PDI	Zeta potential (mV)	%Yield	%EE
GZ-MS1	50.18 ± 1.15	0.62 ± 0.11	-31.12 ± 2.16	97.45 ± 1.83	92.67 ± 1.91
GZ-MS2	57.21 ± 1.80	0.74 ± 0.23	-33.15 ± 2.67	91.30 ± 2.44	87.32 ± 2.13
GZ-MS3	64.12 ± 2.18	0.68 ± 0.32	-36.10 ± 3.14	85.12 ± 2.90	83.43 ± 2.65

Where, PDI: Polydispersity index; %EE: Percentage drug entrapment efficiency.Values were represented as (Mean ± SD) (n = 3).

Higuchi's and Korsmeyer-Peppas to predict their release behaviour. Results of kinetic models of GZ-MS1-3 were given in Table 3. The best fit kinetics, such as Higuchi square root (r^2 =0.941) and Korsmeyer-Peppas (R^2 =0.907) were achieved with GZ-MS1. In Korsmeyer-Peppas model, GZ-MS1 showed (n = 1.127) diffusion exponent as compared to the other formulations.

Stability Analysis

Stability analysis of optimized preparation (GZ-MS1) was conducted according to the ICH guidelines. Sampling was made to check their residual drug content, particle size, zeta potential and physical appearance at the interval of 0, 45, 90 and 180 days. Detailed results of the stability study were shown in Table 4. Result stated that the GZ-MS1 was more stable at 25 ± 2°C when compared with higher temperatures. The rate of degradation (first order) was increased when the temperature was increased due to loss of % drug residual content of the formulation. A plot of (log K) values versus the reciprocal temperature ($1/T\times10^{-3}$) was found to be linear in the selected temperature range (25-40°C). First order and Arrhenius plot of the GZ-MS1 for degradation at 25°C (r^2 = 0.8624), 30°C (r^2 = 0.9970) and 40°C (r^2 = 0.9916) were represented in Figure 6(A,B) respectively. Shelf-life (T_{90}) of GZ-MS1 at 25, 30 and 40°C, GZ-MS1 was found to be 3.79, 2.87 and 1.17 years respectively.

Antioxidant Marker Enzymes Estimation in Rat Gastric Tissue

The levels of rat gastric antioxidant markers were elevated more in group II as compared with the normal group ($^{***}P < 0.001$) which has been shown in Figure 7. The improved levels of gastric antioxidant enzyme systems like CAT, SOD and GPx were significantly achieved with test group III ($^{**}P < 0.05$) and group IV ($^{***}P < 0.001$) when compared to disease control. In case of TBRAS, its altered level was reduced significantly with GZ200 ($^{**}P < 0.05$) and GZ-MS1 group ($^{***}P < 0.001$) in comparison with group II. But the placebo group did not produce any significant effect on the antioxidant enzyme systems of stomach as compared with group II which may be due to the absence of an active moiety (GZ) in the formulation. The detail results were represented graphically in Figure 7(A-D).

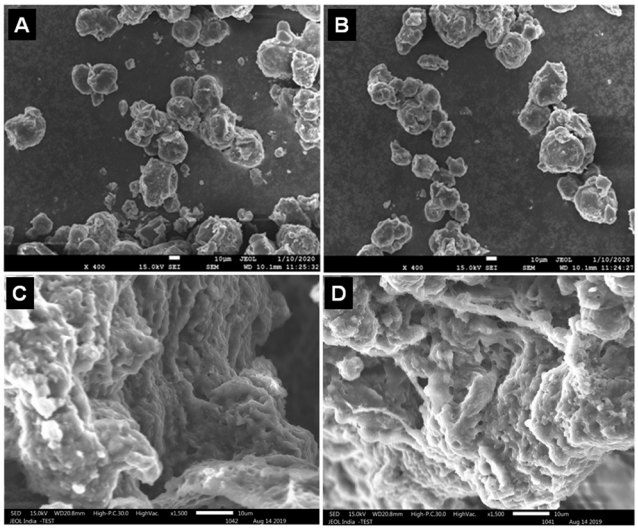

Figure 5: SEM photomicrographs of GZ-MS1 at 400x (A), placebo formulation at 400x (B), GZ-MS1 at 1500x (C) and placebo formulation at 1500x (D).

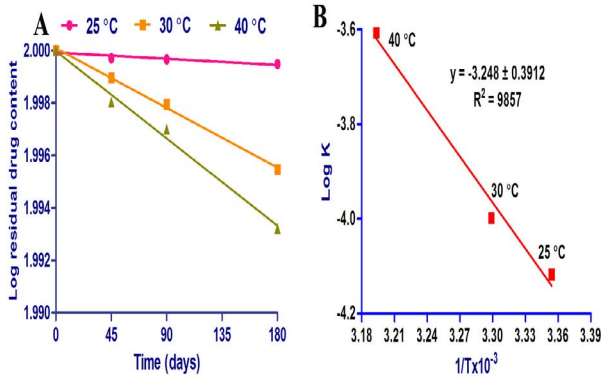

Figure 6: First order (A) and Arrhenius plot (B) of GZ-MS1 for degradation at different storage temperatures (25 °C, 30 °C and 40 °C).

Table 3: Kinetic models of various formulations, GZ-MS1-3.

Formulations	Zero order		First order		Higuchi square root		Korsmeyer peppas	
	r^2	K	r^2	K	r^2	K	r^2	n
GZ-MS1	0.940	4.376	0.970	0.050	0.941	22.32	0.907	1.127
GZ-MS2	0.949	4.005	0.985	0.036	0.936	20.29	0.935	1.173
GZ-MS3	0.961	3.648	0.988	0.028	0.931	18.31	0.963	1.25

DISCUSSION

Emulsification-cross linking technique was used to develop glycyrrhizin-loaded different formulations, GZ-MS1-3 due to its simplicity, reproducibility and ease of handling. Based on GZ and a polymer ratio (1:10-1:16 w/w), GZ-MS1-3 was prepared for gastric delivery against PU. Tiny particle size and negative zeta potential was attributed towards GZ-MS1 which may be due to uniform distribution of particles (0.62 ± 0.11 PDI) with a suitable drug and polymer ratio. The less PDI indicates the homogeneity of the system.[31] When the drug-polymer concentrations were increasing, the characteristics (particle size, zeta potential and PDI) of the formulations (GZ-MS2 and GZ-MS3) were also increased. High %EE was credited to the optimized formulation, GZ-MS1, which may be due to its smaller particle size than the others.

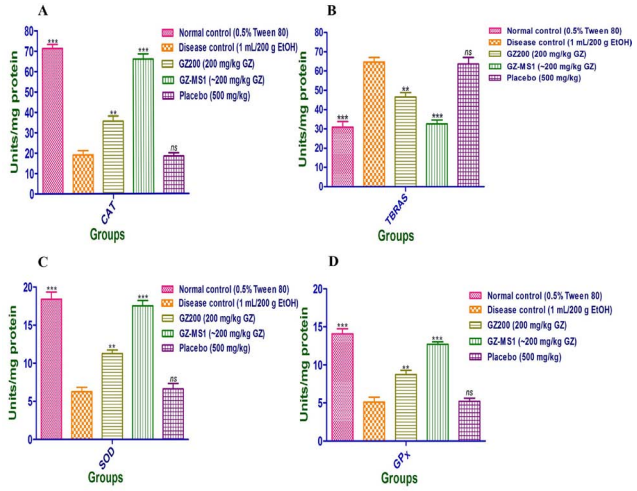

Figure 7: Effect of GZ loaded mucoadhesive formulation on various gastric antioxidant enzyme levels (A) CAT, (B) TBARS, (C) SOD and (D) GPX. Values were shown as Mean ± SEM (n = 6). *P* value (*P* < 0.05 and ****P* < 0.001) as compared to the normal and disease control group.**

GZ-MS1 exhibited a significant degree of swelling and mucoadhesive property at pH 1.2 (SGF) when compared with other formulations. The mucoadhesiveness with stomach lining containing glycoproteins (mucins) favors controlled release of the formulation and helps in retaining it for longer periods. Mucoadhesion offers electrostatic interaction that includes Vander Waal forces and hydrogen bonding between mucin network and bioadhesive polymers.[32-35]

The *in vitro* dissolution study was performed for 24 h to ensure the sustained release properties of the developed mucoadhesive delivery systems. The order of drug release of the different formulations (GZ-MS1-3) were obtained in the sequence of GZ-MS1>GZ-MS2>GZ-MS3. GZ-MS1 showed more sustained release profile for 24 h in comparison with GZ-MS2 and GZ-MS3 which may be due to the smaller particle size and greater mucoadhesive efficiency. The GZ-MS1 expressed its release kinetic in a non-Fickian pattern (super Case-II transport mechanism). These could be due to matrix erosion and the diffusion of the formulation.[36]

Stability study indicated that GZ-MS1 was more stable at the room environment (25°C) rather than the elevated temperature because when the storage temperature was changed towards higher temperature (40°C), their zeta potential was also changed. Products stability was linked

Table 4: Stability studies of optimized preparation at different storage conditions according to ICH guidelines.

Temperature	Periods (in days)	%Residual drug content	Log residual drug content	Particle size (µm)	Zeta potential (mV)
25 ± 2°C / 60 ± 5 % RH	0	100	2	50.18 ± 1.15	-31.15 ± 2.16
	45	99.93	1.99969	50.56 ± 1.20	-31.67 ± 1.98
	90	99.92	1.99965	51.85 ± 1.33	-31.90 ± 1.36
	180	99.88	1.99947	52.14 ± 2.10	-32.81 ± 1.11
30 ± 2°C / 75 ± 5 % RH	0	100	2	50.18 ± 1.15	-31.15 ± 2.16
	45	99.76	1.99895	51.75 ± 1.84	-31.85 ± 2.45
	90	99.53	1.99795	52.28 ± 2.11	-32.98 ± 1.78
	180	98.46	1.99325	53.95 ± 2.18	-33.08 ± 1.12
40 ± 2°C / 75 ± 5% RH	0	100	2	50.18 ± 1.15	-31.15 ± 2.16
	45	99.55	1.99804	52.66 ± 2.45	-36.35 ± 1.21
	90	98.31	1.99699	58.74 ± 1.86	-38.64 ± 1.77
	180	97.45	1.99321	65.18 ± 1.53	-41.30 ± 1.56

with their zeta potential; result stated that negative zeta value was attributed for GZ-MS1 which sustains its stability for longer storage conditions. Zeta potential could be a major stability factor for the formulations.[37] The pure glycyrrhizin (GZ200) exhibited less antioxidant potential through different gastric enzymes as compared with GZ-MS1. GZ-MS1 exhibited improved antioxidant potential which may be due to its enhanced gastric absorption.[38] Hence, glycyrrhizin loaded mucoadhesive microspheres could be promising for gastric delivery.

CONCLUSION

The oral controlled release system of GZ was developed with carbohydrate polymers (SA and GG). The optimized GZ-mucoadhesive microspheres exhibited enhanced drug absorption and gastroprotection effect, which might be due to its potent antioxidant potential against oxidative stress induced by ethanol. The GZ-MS1 was found to be an effective, stable and safe for oral delivery. It significantly restores the altered level of various gastric antioxidant enzymes in ethanol induced oxidative stress. Thus, the SA-GG based GZ loaded mucoadhesive microspheres could be explored further as a promising carrier for the treatment of PU.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

CAT: Catalase; **EE:** Entrapment Efficiency; **FTIR:** Fourier Transforms Infrared; **GPx:** Glutathione Peroxidase; **GZ:** Glycyrrhizin; **GZ-MS:** GZ Loaded Microspheres; **ICH:** International Conference on Hormonization; **PU:** Peptic Ulcer; **SEM:** Surface Electron Microscopy; **SGF:** Simulated Gastric Fluid; **SOD:** Superoxide Dismutase; **TBRAS:** Thiobarbituric Acid Reactive Substances.

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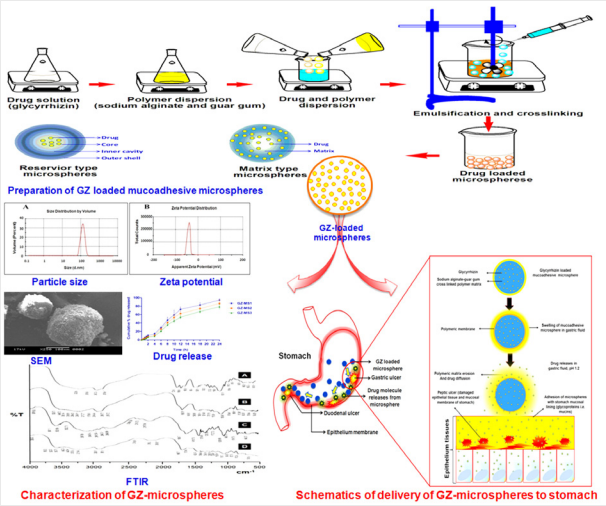
PICTORIAL ABSTRACT



SUMMARY

Glycyrrhizin (GZ) is reported for its antioxidant and gastroprotection potential but its therapeutic efficacy is limited due to low absorption, short half-life and poor bioavailability. Aim of the current study was to formulate sodium alginate and guar gum based GZ loaded mucoadhesive microspheres for the management of peptic ulcer. GZ loaded microspheres (GZ-MS1-3) were prepared by an emulsification-crosslinking technique. Suitable particle size, zeta potential, significant swelling index, mucoadhesive efficiency and maximum drug release profile in simulated gastric fluid (SGF, pH 1.2) was achieved with GZ-MS1. FTIR confirmed that there was no any interaction observed between GZ and excipients. The reduced levels of gastric antioxidant enzymes (catalase, thiobarbituric acid reactive substances, superoxide dismutase and glutathione peroxidase) with respect to the normal group were improved significantly by GZ-MS1 against ethanol-mediated oxidative stress ($^{**}P < 0.05$ and $^{***}P < 0.001$). Thus, the mucoadhesive microspheres of GZ could be an effective strategy for the management of peptic ulcer.

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