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Formulation and *In Vivo*, *In Vitro*, *Ex-Vivo* Evaluation of Gallic Acid Loaded Floating Mucoadhesive System

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Abstract

Despite major breakthroughs in therapy over the past few decades, peptic ulcer disease has remained a prevalent disease. The objective of this research was to formulate Gallic acid loaded floating mucoadhesive microsphere for treating peptic ulcer by full factorial design approach. Two factor three level factorial design was used for optimisation of formulation. GA loaded microsphere was developed by ionic gelation method using sodium alginate and calcium chloride. Various Polymer and crosslinking agent were screened out of which Sodium bicarbonate, Eudragit L100 and Guar gum were selected for development of formulation. Drug: Polymer ratio, stirring speed were selected as two independent factors. These two factors were investigated at three levels. Entrapment efficiency and percent drug release were dependent factors and satisfactory results found for all experimental batches. Amongst all the experimental batches an optimized batch was obtained and further characterized for micromeritic properties, SEM analysis, DSC analysis, in-vitro dissolution studies, anti-microbial activities, antioxidant assay, ex-vivo mucoadhesion. The further in-vivo evaluation showed that animals treated with an optimized formulation with gallic acid showed significant inhibition of peptic ulcers. Stability study of optimized formulation showed that tablets were stable at 25±2℃ and 5±3℃. It was concluded that floating mucoadhesive microsphere containing Gallic acid could be a promising strategy for treating peptic ulcers.

Keywords

Gallic acid, Gastric ulcer, Microsphere, Floating -Mucoadhesion, Antimicrobial, Antioxidant.

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INTRODUCTION

Treatment of upper gastrointestinal tract (GIT) infection is challenging due to the location of the infection site in stomach mucus lining. GIT is divided into three main parts: stomach, small intestine (duodenum, jejunum and ileum), and large intestine. Because there are two patterns of gastrointestinal (GI) motility and secretion, one for fasted and one for fed states, the outcomes of orally delivered medications will vary depending on the state of meals. Fasting is linked to a series of cyclic events known as the migrating motor complex (MMC), which control GI motility patterns. The MMC is organised in an alternating activity cycle that can be categorized into three phases: basal (Phase I), preburst (Phase II), and burst (Phase III). Phase-I: This is a phase of no contractions that lasts 30 to 60 minutes. Phase-II: This phase lasts roughly 20-40

minutes and comprises of infrequent contractions that gradually increase in intensity as the phase advances. Later in the phase, gastric release of fluids and exceptionally small particles commences. Phase-III: This is a brief phase of severe distal and proximal gastric contractions (4-5 contractions per minute) that lasts 10-20 minutes; these contractions, also known as "House- keepers wave" sweep gastric contents down the small intestine. Phase-IV: Between the last part of phase III and the quiescence of phase-I, there is a transitory period of roughly 0 to 5 minutes during which the contraction dissipates.[1] Ulcers are lesions on the stomach or small intestine lining. Lesions can also occur in the oesophagus (throat), small intestine, and stomach; however, most ulcers occur in the stomach. These ulcers are called gastric ulcers. The bacteria Helicobacter pylori (H. pylori) and long-term use of nonsteroidal anti-inflammatory medicines (NSAIDs) such as ibuprofen (Advil, Motrin IB, others) and naproxen sodium (Aleve), stress, smoking, alcohol, and spicy food are the most prevalent causes of ulcers.[2] Following are the different types of ulcers on the basis of their specific location such as:

Gastric ulcer (GU): This type of ulcer found in the stomach. It is one of the most common digestive system disorders, with a high morbidity of around 5–10% over the course of a person's lifetime, making it a major public health burden in the twenty-first century. Although the etiology and pathogenesis of GU remains controversial, numerous studies have revealed that it is caused by the critical imbalance between mucosal invasive factors (such as long period consumption of nonsteroidal anti-inflammatory drugs) and the protective factors of gastric mucosa (especially prostaglandins level and antioxidant enzymes activity), resulting in disruption of the gastric mucosal defensive barrier thus leading to gastric ulcer. Duodenal ulcer (DU),Esophageal ulcer, Meckel's diverticulum Ulcer, Prepyloric ulcer and Proximal gastro esophageal ulcer are different ulcers based upon the site.[3]

Treatment of local gastric infection with conventional formulations becomes ineffective due to their short gastric residence time and non-targeted drug release. Gastric emptying, which is highly variable, transfer the conventional formulation quickly to the intestine without significant release of drug to the mucous site. Thus frequent dosing is required.[4,5] For example Misoprostol is absorbed systemically, but need for four times daily dosing limit the duration and degree of exposure.[6] Gastroretentive drug delivery system helps to improve therapeutic efficacy and bioavailability which may result in decrease in the dosing frequency of the dosage form.[7] Gastroretentive drug delivery system developed as a drug delivery system for better eradication of GI infection.[8] Microencapsulation is a useful method for prolonging drug release from dosage form and reducing adverse effect.[9] Recently, dosage forms that can precisely control the release rates and target drugs specific body site have made an enormous impact in the formulation and development of novel drug delivery system. Microspheres form an important part of such novel drug delivery system.[10–12] Microspheres are one of the Multiparticulate delivery systems and are prepared to obtain prolonged or controlled drug delivery to improve bioavailability or stability and to target drug to specific sites. Microspheres can also offer advantages like limiting fluctuation within therapeutic range, reducing side effects, decreasing dosing frequency, and improving patient compliance.[13,14]

Gallic acid (3, 4, 5-tripHydroxyl-benzoic acid) is polyphenolic acid. Polyphenol' are compounds that carry more than one phenol group. They are the largest group of secondary metabolites of plants. Polyphenols are powerful antioxidants that complement and contribute to the function of antioxidant vitamins and enzymes as a defense against oxidative stress caused by reactive oxygen species (ROS). GA is a polyphenol, found particularly in red fruits such as strawberries, grapes, bananas, pineapples, lemons, apple peels, food such as pigeon trees, chocolate and wine[15–17]. GA is a strong antioxidant, cyclooxygenase and lipoxygenase inhibitor and apoptosis inducer with a vasoconstrictor effect[18]. It is also used as a antibacterial agent GA had a higher activity, against gram-positive (*S. epidermidis* and *S. aureus*) and gram-negative bacteria (*K. pneumoniae*) at lower concentrations, than the other compounds.[19]

Gastroretentive drug delivery systems favor prolonged drug release in the stomach.[20] Unlike traditional controlled release formulations, they bypass the gastric emptying process which interferes with drug delivery to the upper GIT.[21] Gastroretentive drug delivery systems have been developed employing floating technique. Ritesh Kumar et. al developed HPMC based GA floating microsphere and showed gastric buoyancy for a prolonged period.[22] In general, these floating dosage forms release drugs at multidirections and cannot selectively release drugs on the mucosal surface. Consequently, small amount of drug reaches the target site from multidirectional drug release. Release of drug to the specific site is important for effective treatment.[23] It is particularly necessary

for eradicating the local infection at the mucous layer where drugs from conventional formulations may not reach.[24] Previous studies mainly focused on developing floating formulations. However, site specific GA delivery system was not explored. Mucoadhesive drug delivery systems have recently been explored for sustained release at the mucosa and increasing bioavailability of drugs.[25] Indeed, drug delivery systems with floating and mucoadhesive properties may ideally maximize drug release to the specific site, adhering to the mucous layer, and in treatment of upper GIT infection.[26]

This study was aimed to develop GA loaded floating-mucoadhesive microsphere for drug release at the mucous layer of upper GIT. GA microsphere was developed by ionic gelation method using sodium alginate and calcium chloride. Sodium bicarbonate was used to incorporate floating property in the microsphere. Eudragit L100, which dissolves at a pH greater than 6, and was used for sustained drug release. Guar gum, a natural viscous polymer having adhesive property, was used for mucoadhesion. Additionally, guar gum was reported to be useful as a gastroprotective agent against peptic ulcer. It reduces gastric acid, and promotes ulcer healing.[27] Besides, alginate has good mucoadhesive property.

MATERIAL AND METHOD

Materials:

Gallic acid was obtained as gift sample from twinkle chemi lab Pvt Ltd. (Maharashtra, India), Carbopol, calcium chloride and Eudragit was purchased from S. D Fine Chem. Ltd. Sodium Alginate was purchased from SRL. glacial acetic acid was purchased from Loba chemie Pvt Ltd, Sodium bicarbonate was purchased from Loba chemie Pvt Ltd, Methanol AR was purchased from S.D. Fine chem. Ltd. Male Wistar rats been used with permission of IAEC in the proposal no. IAEC-BCP/2020-02/09.

Screening of Excipients:

Polymer and crosslinking agent were screened based on trial batches, in which polymers such as Eudragit L100, HPMC K-400, Ethyl Cellulose, Carbopol, Chitosan, HPMC K-100, Eudragit S-100, Sodium Alginate was screened and for the crosslinking agent, Barium chloride, Aluminum chloride, Glutaraldehyde, Calcium chloride was screened based on the yield. Polymers enable the production of uniformly shaped and well- defined spheres hence a choice of compatible polymer is utmost important factor for microsphere production. The hardening of the microsphere is dependent on the crosslinking reagent. Hence crosslinking agents were scanned and desired hardening providing agent was chosen.

Preparation of floating mucoadhesive gallic acid loaded microspheres:

A GA floating-mucoadhesive microsphere was prepared by ionic gelation method varying the polymer ratio and stirring speed. Sodium alginate was dissolved in deionized (DI) water. Carbopol was dissolved separately in DI water, and Eudragit L100 was mixed in the thick Carbopol solution then guar gum was added to the Eudragit-Carbopol mixture. GA was added in the Carbopol-Eudragit matrix and stirred vigorously. Then the gas forming agent sodium bicarbonate was mixed. The prepared slurry was added to sodium alginate solution and mixed continuously. Crosslinking solution was prepared by dissolving calcium chloride in DI water containing glacial acetic acid. Then the mixture, free from air bubbles, was added dropwise to the crosslinking solution through a syringe containing 26G needle. The immediately formed beads were collected by filtration and air dried for 8-10 hours.

Optimization by Full factorial design:

The formula for floating-mucoadhesive microsphere preparation was optimized by two factor-three level factorial design using Design Expert software ® (Version 13.0.5.0 Stat Ease, Minneapolis, MN). Drug: Polymer ratio (A), Stirring speed (B) were selected as two independent factors. These two factors were investigated at three levels: low (-1), middle (0) and high (+1). Entrapment efficiency (Y1) and percent drug release(Y2) were the response that were recorded. The constraints applied were high entrapment efficiency and high percent drug release. The factorial design gave 9 runs. Entrapment efficiency and percent drug release of all runs were observed.

Characterisation of floating mucoadhesive gallic acid loaded microspheres:

Entrapment efficiency:

Microspheres, containing 200 mg of GA, were crushed and immersed into 100mL of simulated gastric fluid SGF (0.1N HCl pH 1.2). The suspension was kept oscillating overnight and filtered. The drug concentration was determined by a UV spectrophotometer (V -1900, Shimadzu, Japan) at the wavelength of 271 nm.

$$\%\mathbf{DEE} = \frac{\textbf{Actual drug content}}{\textbf{Theoretical drug content}} \times \mathbf{100}$$

In-Vitro buoyancy percentage:

In-vitro floating properties of the GA loaded microspheres were evaluated in a USP dissolution apparatus II (paddle type, Lab India DS 800). 50 individual microspheres from each formulation were immersed into the vessel filled with 500 mL of SGF. The paddles were rotated at 50 rpm and the temperature was maintained at 37±0.5°C. The

number of floating microspheres was counted at hourly intervals up to 12 hours.

Swelling Index:

Swelling study was conducted using the dissolution test apparatus II. Accurately weighed amount of beads were placed in the vessels containing SGF and allowed to swell. Rotation speed was set at 50 rpm. The microspheres were withdrawn at predetermined time intervals and blotted with filter paper to remove excess amount of water. The changes in weight were measured at different time intervals until maximum weight was gained.

$$s = \frac{\mathrm{Wm} - \mathrm{Wt}}{\mathrm{Wt}}$$

Where, W_t denotes the initial weight of the microspheres, Wm denotes the final weight

In vitro drug release study:

Drug release from the floating microspheres was investigated using the USP dissolution apparatus II (paddle type). SGF (0.1N HCl; pH 1.2) was used as the dissolution medium and 900mL of it was poured into each dissolution vessel. Microspheres, equivalent to 200 mg of Gallic acid were placed inside the baskets and they were rotated at a speed of 100 rpm, maintained at a temperature of 37±0.5°C. An aliquot of 5mL was withdrawn at predefined intervals up to 12 hours and the volume was replaced with 5mL of fresh medium. The aliquots were diluted and the concentration of gallic acid was determined spectrophotometrically at 271 nm.

Micromeritic properties:

Bulk density:

The bulk density was performed using the three-tap method and it is obtained by dividing the weight of the sample in grams by the final volume in cm^3 of the sample contained in the cylinder.

$$\textbf{Bulk density} = \frac{\textbf{Mass}}{\textbf{Bulk volume}}$$

Tapped density:

The tapped density of a powder is the ratio of the mass of the powder to the volume occupied by the powder after it has been tapped for a defined period of time. The tapped density of a powder represents its random dense packing. Tapped density can be calculated as follows

$$\textbf{Tapped density} = \frac{\textbf{Mass}}{\textbf{Tapped volume}}$$

Angle of repose:

Angle of repose (Θ) of microspheres measures the resistance to particles flow and is calculated according to fixed funnel standing cone method. The angle of repose is measured by the inverse tangent (arctan) rule at which the average radius of the formed conical shape and the maximum height of the heaped material are measured, and then the angle of repose is determined as the arctan of the maximum height to average radius ratio. It is given as:

$$\boldsymbol{angle\ of\ repose}(\Theta) = \mathbf{tan^{-1}}(\boldsymbol{h/r})$$

Carr's Index:

It is an indication of the compressibility of the powder and is calculated by the formula given below.

$$\boldsymbol{carr's\ index} = \frac{\boldsymbol{\rho tapped} - \boldsymbol{\rho bulk}}{\boldsymbol{\rho tapped}} \times \mathbf{100}$$

Where, ρ_{tapped} is the tapped density and ρ_{bulk} is the bulk density

Hausner's ratio:

It is number that is correlated to the flowability of a powder or granular material here,microspheres. It is given by the formula:

$$\boldsymbol{Hausner's\ ratio} = \frac{\boldsymbol{\rho tapped}}{\boldsymbol{\rho bulk}}$$

Where, ρ_{tapped} is the tapped density and ρ_{bulk} is the bulk density

Differential Scanning Calorimetry:

The thermal characteristics of Gallic acid were analysed using Differential Scanning Calorimetry (DSC). The DSC thermogram of pure Gallic acid was obtained using DSC (DSC STARE system, Mettler Toledo, Switzerland), equipped with Intercooler 2P cooling accessory. About 10mg of Gallic acid loaded microspheres was weighed and filled in DSC pan and sealed properly. Then this pan was placed in DSC instrument along with reference pan and heated from 30°C to 300°C. The heating rate of pan was maintained at 10°C/min. The nitrogen gas is purged at the rate of 20 mL/min during experiment to maintain inert environment and endotherm was recorded.

Scanning Electron Microscopy (SEM):

SEM micrographs of microspheres were obtained under high resolution (Mag 500X, 15kv) Using JSM-6100, scanning electron microscope (SEM), equipped with a digital image processor. It has a large specimen chamber that allows observation of the entire surface of a specimen upto 150 mm and a tilt of -5 to 90^o. SEM micrographs were taken at Panjab university, Chandīgarh, India.

Ex-vivo muco-adhesion study:

A strip of rat stomach mucosa 1 cm × 1 cm was mounted on a glass slide and accurately weighed microspheres were placed on the tissue kept in a desiccator at 90% relative humidity for 15 min to allow the microspheres to interact with the membrane and by fixing at an angle of 45° relative to the horizontal plane. SGF (pH 1.2) was peristaltically pumped at a rate of 2 ml/min over the tissue. The washings were filtered and dried.

$$\boldsymbol{Percentage\ mucoadhesion} = \frac{\mathrm{Wo} - \mathrm{Wt}}{\mathrm{Wo}}$$

Where, Wo = weight of microspheres applied, Wt = weight of microspheres leached out

Anti-oxidation study:

The DPPH radical-scavenging activity was determined; DPPH (0.1 mM) was dissolved in pure ethanol (96%). The microsphere stock solution was prepared freshly. The DPPH solution (1 ml) was added to different concentrations of isolated sample with 3 ml of ethanol. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 10 min. The decrease in absorbance of the resulting solution was monitored at 517 nm. The results were corrected for dilution and expressed in % inhibition. Equal volume of ethanol & DPPH was used as control. All determinations were performed in triplicate.

Histopathology study:

The gastric tissues were fixed in neutral buffered formalin solution for duration of 24 hrs. Sections of tissue from stomachs were examined histopathologically to study the ulcerogenic and/or anti-ulcerogenic activity of GA microspheres. These sections were stained with hematoxylin and eosin after treatment. The slides were examined microscopically for pathomorphological changes such as congestion, hemorrhage, oedema, and erosions using an arbitrary scale for the assessment of severity of these changes.

***In vivo* ulcer curative study:**

Adult albino wistar male rats weighing about 150–200 g maintained under standard conditions of temperature, humidity, and light. Food and water were provided ad libitum. The study was approved by the Institutional ethical committee, which follows the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals, Reg. No. 242/PO/RE/S/2000/CPCSEA), Government of India, New Delhi, India.

Adult albino wistar male were divided into four groups of six animals each. A group of mice was treated orally once daily with aspirin (300 mg/kg) for 5 consecutive days to induce ulcer.

- Group I: Animals were given neither aspirin nor formulation. This group served as control.
- Group II: Animals were given with 300 mg/kg of aspirin. This group served as positive control.
- Group III: Animals were treated with 300 mg/kg of aspirin and famotidine. This group
- served as standard treatment group
- Group IV: Animals were treated with 300 mg/kg of aspirin and 200mg/kg of formulation. This group served as treatment group.

On day 6, Animals were sacrificed under CO_2 Chamber. The stomach was excised and opened along the greater curvature for lesions. Lesions severity was determined by ulcer index.

The mean ulcer score for each animal will be expressed as ulcer index.

Ulcer index= [Ulcerated area/total area of stomach] × 100.

The percentage of ulcer protection was determined as follows:

$$\%\ \boldsymbol{protection} = \frac{(\boldsymbol{control\ mean\ ulcer\ index}) - (\boldsymbol{test\ mean\ ulcer\ index})}{\boldsymbol{control\ mean\ ulcer\ index}} \times 100$$

Stability studies:

The Stability of Gallic acid microspheres were checked to assess the long-term usability of formulation. The stability study of formulation gives us idea about potential excipient reaction, long term drug stability and possible drug expulsion from formulation. It also assesses the stability of formulation at different environment and storage condition. The preparation was divided into three sets and was stored at 4℃, room temperature (25℃) and 40℃ (thermostatic oven). Formulation was tested at 0, 30, 60 and 90 days. The formulation was tested for floating ability, entrapment efficiency and % drug release by the method discussed earlier.

RESULT AND DISCUSSION:

Screening of excipients:

Sodium alginate was selected as a mucoadhesive agent, Eudragit L-100 as a release modifier, Carbopol to increase bead strength and Calcium chloride as a crosslinking agent. Table I shows the composition of the trial batches that were designed by the software.

Table I: Composition of the trial batches (F1 to F9)

Quantities in mg	Batches								
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Gallic Acid	200	200	200	200	200	200	200	200	200
Carbopol	180	180	180	180	180	180	180	180	180
Eudragit L-100	150	150	150	150	150	150	150	150	150
Sodium bicarbonate	600	600	600	600	600	600	600	600	600
Calcium chloride	2500	2500	2500	2500	2500	2500	2500	2500	2500
Glacial acetic acid (ml)	5	5	5	5	5	5	5	5	5
Guar gum	500	500	500	500	500	500	500	500	500
Sodium alginate	**3000**	**1500**	**3000**	**1500**	**3000**	**750**	**750**	**1500**	**750**
Stirring speed	**1000**	**800**	**800**	**1200**	**1200**	**1000**	**1200**	**1000**	**800**
Production yield %	**72.33**	**83.30**	**89.95**	**74.44**	**74.17**	**63.63**	**60.6**	**82.57**	**74.87**
%Entrapment Efficiency	**70**	**67**	**73**	**60**	**67**	**52**	**50**	**62**	**58**
% Drug release	**92.27**	**81**	**85**	**73**	**88.3**	**68**	**66**	**93.89**	**77**

Table II: Optimization of gallic acid loaded floating mucoadhesive microsphere

Runs	Drug: polymer (Sodium alginate)	stirring rate	Entrapment efficiency (Y1)	% Release (Y2)
1	1	0	70	92.36
2	0	-1	67	81
3	1	-1	73	85
4	0	1	60	73
5	1	1	67	88.43
6	-1	0	52	68
7	-1	1	50	66
8	0	0	62	93.97
9	-1	-1	58	77

Where -1 is 1:1, 0 is 1:7.5 and 1 is 1:15 for drug: polymer ratio while -1 is 800rpm, 0 is 1000rpm and 1 is 1200rpm

Optimization of floating mucoadhesive gallic acid loaded microsphere:

The trail runs were prepared according to the design designed by the software and following are the responses observed as mentioned in **Table II**. Amongst all models, Quadratic model was best fitted models for all two independent variables. An optimized run was selected from the given solution for constrains which was then formulated and characterized.

Equations for gallic acid loaded floating mucoadhesive microspheres optimization:

The equation for each response was calculated by least square regression method using Design expert statistical software. The equation for all responses is given below.

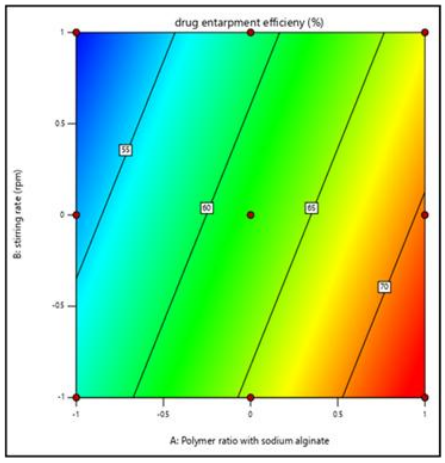
Drug entrapment efficiency (Y1) = 62.1111 + 8.33333 *drug: polymer ratio+-3.5 * Stirring rate

%Drug release(Y2) = 80.5289 9.13167*drug: polymer ratio + -2.595 * Stirring rate

Effect of variable on Drug entrapment efficiency:

The Entrapment efficiency for optimization batches were in the range of 50-73%. The minimum entrapment efficiency was was 50% oF F7 whereas the maximum efficiency was 73% of F3. It shows entrapment efficiency is increased due to an increase in the concentration of sodium alginate. The entrapment efficiency depends on the type and amount of polymers used. It was found that, if increasing the amount sodium alginate, the entrapment efficiency was increased. As the stirring rate increase entrapment efficiency decreases. The ANOVA for selected linear model for Entrapment efficiency was analyzed. According to the analysis for entrapment efficiency, the p-value for model terms A and B was less than 0.0001 and p-value below 0.05 indicates the model terms are significant. Hence the mentioned model terms are significant. The R^2 was found to be 0.9825. The standard deviation was 1.21 and mean was 62.11.

The contour plot and 3D surface response graph are shown in **Figure 1 (a) and (b)**respectively.



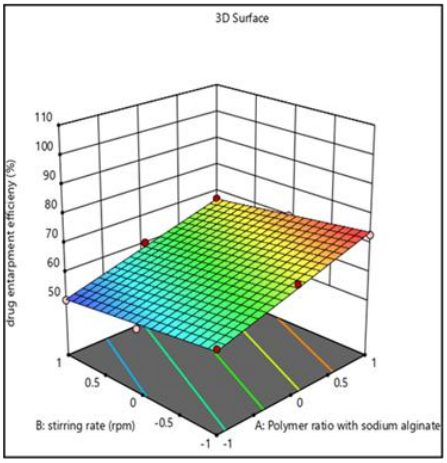
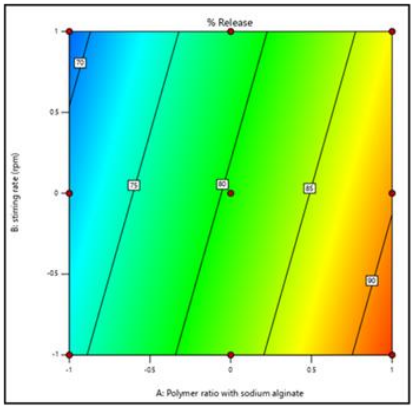


Figure 1: (a) contour plot for entrapment efficiency **(b)** 3D surface response graph for EE



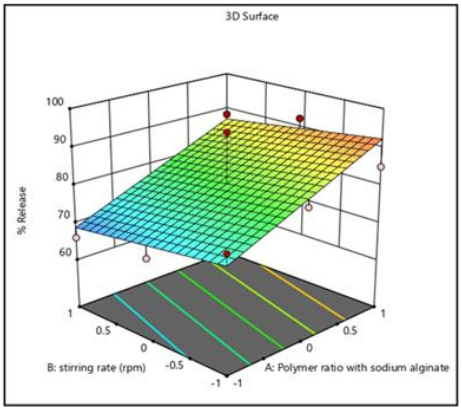


Figure 2 (a) contour plot for drug release **(b)** 3D surface response graph for drug release

Effect of variable on percent drug release:

The Percent In-Vitro drug release by USP dissolution testing apparatus II (Paddle type, lab India DS800) was found to be in the range of 66-93.97%. The minimum release was 66% of F7 and maximum was 93.97%. of F8 According to the analysis for In-Vitro drug release, the p-value for model terms A was less than 0.0001 indicating the model terms are significant. Hence the mentioned model terms are significant. The R^2 was found to be 0.6434.

The contour plot and 3D surface response graph are shown in **Figure 2 (a) and (b)**respectively.

From the above study an optimised batch was obtained it was prepared by the same ion gelation method. The formula for optimised batch was obtained with the optimal value for Drug:polymer ratio of 1:15 and Stirring speed of 800 rpm.

Characterisation of the optimised batch:

Entrapment efficiency:

Entrapment efficiency of the optimized formulation was found to be 73.6 ± 0.6%.

***In-Vitro* buoyancy percentage:**

Buoyancy percentage of the optimised formulation was found to be 81 ± 0.01%

Swelling Index:

Swelling index of the optimized formulation was found to be 57.16 ± 0.76%.

In vitro drug release study:

In-vitro drug release of the optimised formulation was found to be 92.24 ± 0.39%**.** Kinetic models for invitro release study of gallic acid loaded floating mucoadhesive microsphere were evaluate the kinetic models illustrate the factors involving in dissolution of drug from formulation the regression coefficient l.e R^2 of various kinetic models was obtained by plotting the data in graphs. The R^2 value of korsmeyer peppas model for gallic acid loaded floating mucoadhesive microsphere is 0.9893. Hence this indicates that korsmeyer peppas model has best linearity than other models.

Micromeritic properties:

The micromeritic properties of optimized batch is shown in **Table III.**

Table III- Micromeritics properties of optimised batch

Evaluation Parameter	Results
Angle of repose (Degree)	28^{o}±0.57
Bulk Density (g/cm^3)	0.46±0.005
Tapped Density (g/cm^3)	0.54±0.017
Carr's Index	14.14±2.4
Hausner's ratio	0.88±0.005

Differential Scanning Calorimetry (DSC):

The thermal analysis of GA and GA loaded microspheres were studied by differential scanning calorimetry (DSC). The DSC data give us idea about the melting point, crystallinity, and degradation of sample. The DSC thermogram of GA (**Figure 3 (a)**) shows sharp endothermic peak at 264.34^{0}C which indicates the melting point of GA, and narrow peak indicates its crystallinity. The thermogram shows melting endotherm of GA loaded microspheres (**Figure 3 (b)**) shows two peaks.one peak at 126.49^0 C and other at 173.58^0 C. The absence of the endothermic peak at 264.34^{0}C indicated that the drug is incorporated in the matrix of polymer which forms floating mucoadhesive microsphere.

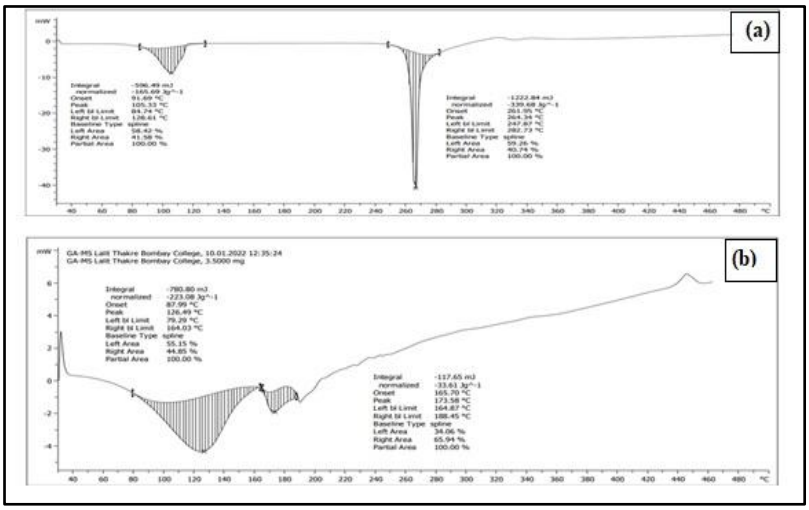


Figure 3: **(a)** DSC of pure Gallic acid **(b)**DSC of GA loaded microsphere

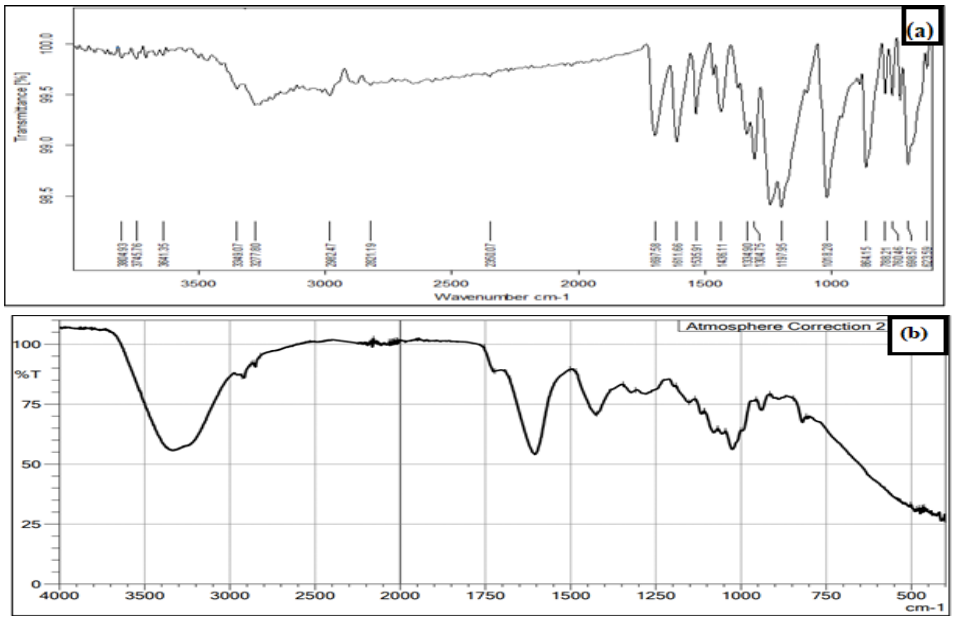


Figure 4: FTIR graph of: (a) gallic acid and (b) GA loaded floating mucoadhesive microspheres

Fourier transform infrared spectroscopy (FTIR) analysis:

Drug polymer interaction was checked by comparing the IR spectra of the physical GA loaded floating mucoadhesive microsphere used with the FTIR spectrum of pure drug. Obtained results shown that there was no possible interaction between drug and polymer. **Figure 4(b)** is the FTIR graph of gallic acid. The principal peaks of Gallic acid were obtained as 3641.35 cm^{-1} of O-H stretch, 3349.07 cm^{-1} of O-H stretch, 1691.49 cm^{-1} of C=O Carboxylic acid stretch. The principal peaks of gallic acid loaded floating mucoadhesive microspheres are 2916.60 cm^{-1} of =C-H Stretch alkene, 2851 cm^{-1} of C-H Stretch alkane, 2163 cm^{-1} of -C=C- stretch alkyne, 1725.72 cm^{-1} of C=O Stretch carboxylic acid, 1426.21 cm^{-1} of C-C stretch (In ring), 1322.10 cm^{-1} of C-O stretch alcohol.

SEM Analysis:

The surface morphology of microspheres represented by the particle size and a characteristic shape was determined by SEM. The SEM images of microspheres taken at different magnifications are shown in **Figure 5.** It was noted in the SEM images that the microspheres were spherical, discrete, and freely flowing. In addition, the surfaces were slightly rough and drug crystals were also present on the surface of the microspheres. These drug crystals were responsible for the burst release of drug from the microspheres.

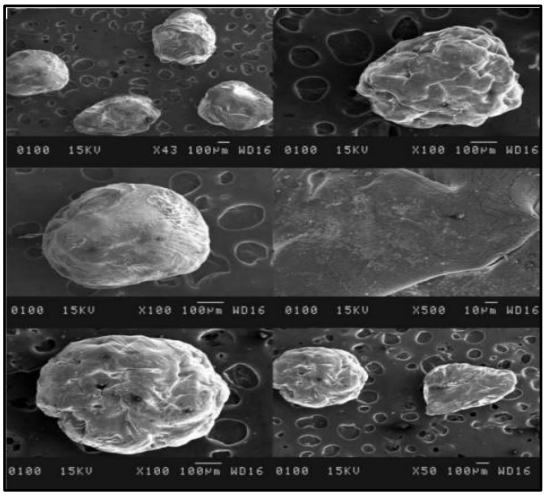


Figure 5: SEM analysis of optimised GA loaded floating mucoadhesive microspheres.

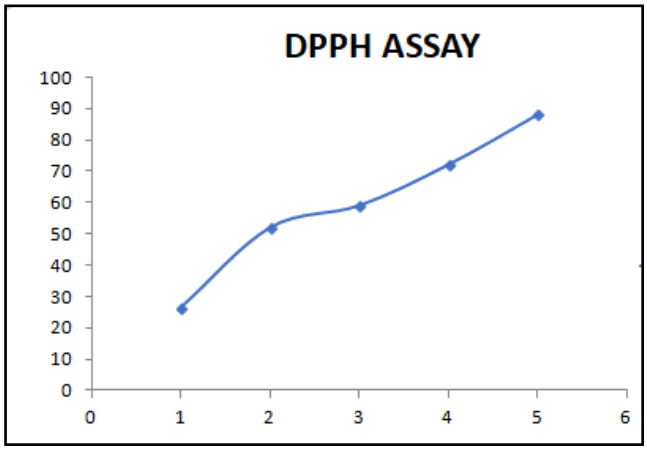


Figure 6: Antioxidant activity of optimised GA loaded floating mucoadhesive microspheres.

Anti-oxidation

The results of in vitro antioxidant activity reveal that sample solution showed marked inhibition in the DPPH assay. It was found that Gallic Acid loaded floating mucoadhesive microsphere extract showed potent free radical scavenging activity even in low concentration. The results are shown in **Table IV.**

Table IV Antioxidation activity

Sr no.	Concentrations (µg mL $^{-1}$)	% Inhibition
1	20	26.43505±0.302115
2	40	52.01415±0.230744
3	60	59.063445±0.302115
4	80	72.205445±0.60423
5	100	88.262845±0.026164

Mucoadhesion study:

Mucoadhesive property of microspheres being explored for targeting purpose is considered as a prime parameter for evaluation of performance as mucoadhesion and its durability both can predict the degree of sustainability and duration of drug availability at the desired site. Microspheres with a coat consisting of sodium alginate and a mucoadhesive polymer exhibited good mucoadhesive properties in the Ex Vivo wash-off test. It was calculated by the formula and found to be **83.1±0.692%**

Animal Study:

This study was performed with permission of Institutional Animal Ethics Committee (IAEC) through approved from B under the project proposal no. IAEC-BCP/2020-02/09 and the work were performed as per the guidelines given by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The results obtained from the study showed characteristic lesions in the glandular portion of rat stomach which appeared as elongated bands of thick, black and dark red lesions. The further evaluation showed that animals treated with an optimized formulation with gallic acid and administered marketed brand of famotidine showed significant inhibition of 91.054%and 84.54% respectively in comparison to control **(Table V)**. Gallic acid is a well-known natural antioxidant that is basically a secondary polyphenolic metabolite. Floating mucoadhesive microsphere of an optimized formulation containing gallic acid showed high ulcer inhibition of 91.054% as compared to 84.54% in the treatment group. It may be due to the effect of gallic acid Macroscopical changes of aspirin-induced models are shown in **Figure 7.**

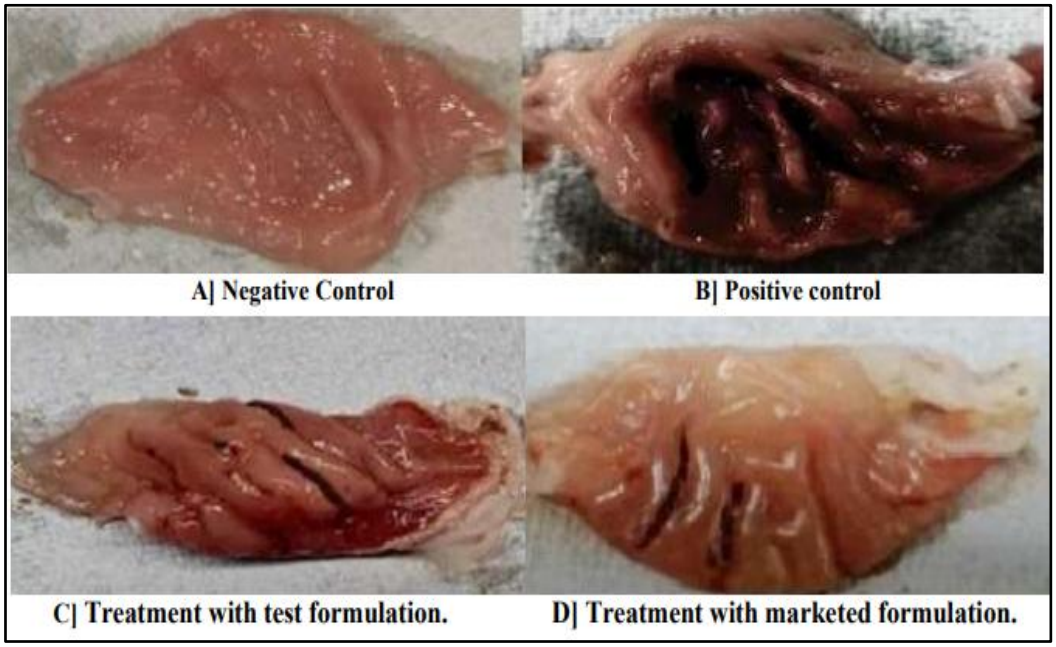


Figure 7: In-vivo antiulcer study of Gallic acid loaded floating mucoadhesive microspheres

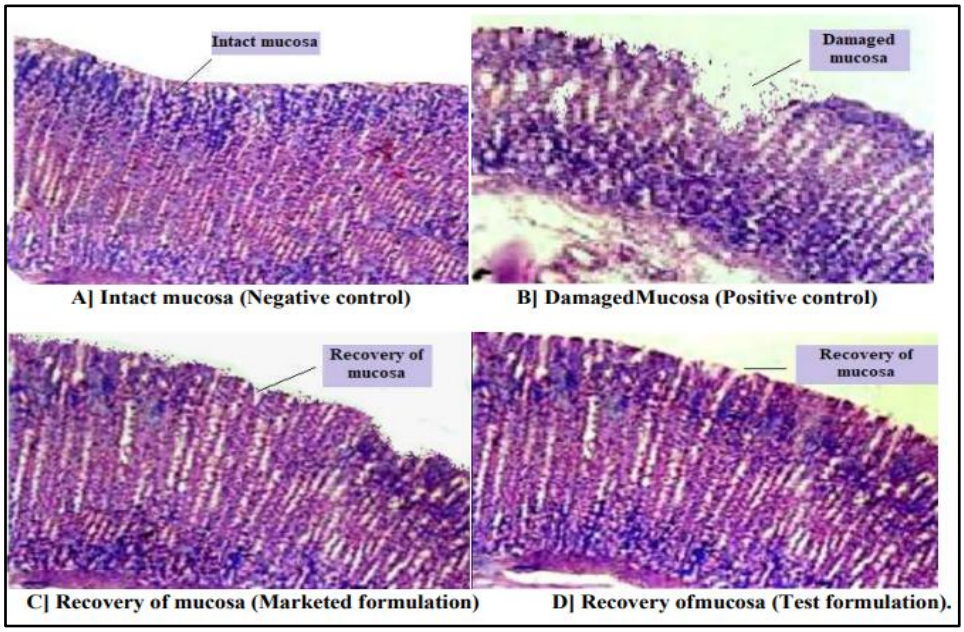


Figure 8: Histopathological examination of Gallic acid loaded floating mucoadhesive microsphere

Table V: *In-vivo* antiulcer activity protocol

Group	Treatment	Ulcer index	Ulcer inhibition (%)
I	Negative control	N/A	N/A
II	Positive control	**23.81**	N/A
III	Std treatment group	**3.68**	**84.54**
IV	Treatment group	**2.13**	**91.054**

Nonsteroidal analgesic and anti-inflammatory drugs like aspirin can effects on oxidant and antioxidant mechanisms and interfere prostaglandin synthesis through cyclooxygenase pathways, produce neutrophils and oxygen radical dependent microvascular injury leading to mucosal damage. Aspirin acts directly by increasing the H+ ion transport while on the mucosal epithelial cells it decreases mucin, surface-active phospholipids, bicarbonate secretion and microvasculature damage by generation of free radicals. Aspirin induced ulceration animals models were used to investigate the antiulcerogenic activity of gallic acid, and ulcer control groups show significant differences in ulcer parameters when compared to healthy control groups. Aspirin induced animals showed extensive gastric lesions. This is evidenced by an increase in ulcer index (UI) when compared to untreated control. Pre-treatment with gallic acid and famotidine produced 91.054% and 84.54% while UI in aspirin treated animals found to be 23.81%.

Histopathological observation also confirmed the ulcer protective effect of gallic acid. GA and famotidine treated group does not showed any ulceration though less inflammation in submucosa can be observed **(Figure 8)**. These observations further support the antiulcer effect of GA. The present results suggest that gallic acid may have in vivo antioxidant and antiulcerogenic effect on gastric lesion induced by aspirin. GA may appear to exert its antiulcer effects by increased mucosal defensive and decreased offensive factors. GA may also appear to activate antioxidant mechanism and inhibit toxic oxidant mechanisms in stomach tissues, which is also responsible for its antiulcer effect

Stability study:

The stability study was performed as per the ICH guidelines in which formulation was said to be stable if its physical and chemical integrity remains intact over a period. After 3 months storage period, there were no change in physical property, colour and no liquefaction were observed. The drug entrapment efficiency was found to be maximum at room temperature (25±2^{o}C). The buoyancy was not changed much (≤5) for the stored formulation. Initially the buoyancy at the start of the study for temperature 5±3^{o}C was 83.86±0.355 which changes to 81.88 ±0.569 after 3 months study (n=3). While at room temperature (25±2^{o}C) it was 82.376 ±1.262 initially which changes to 81.61±1.466 after 3 months respectively. Thus, the sample stored at 5±3^{o}C shows maximum buoyancy which might be due to aggregation of microsphere stored in refrigerated conditions. The size of the particle has

an inverse relationship with density. Hence a slight increase in buoyancy might be due to an increase in aggregation of particle.

No considerable changes are found in the percent drug release from microsphere formulation at different storage conditions. Initially the floating mucoadhesive microspheres at 5±3°C showed 93.22±1.37% drug release in 12 h while at the end of 3 months the formulation showed 85.63±0.98%. While at the room temperature (25±2°C) the release was initially found to be 92.76±1.07 and after 3 months the release was 89.27±1.05 thus the results of the stability studies of the GA loaded floating mucoadhesive microsphere as per the evaluation performed at 25±2°C and 5±3°C were shown that formulation was stable at both temperature conditions but it is most stable at room temperature as per results obtained from the stability study.

CONCLUSION:

We can conclude that floating-mucoadhesive microsphere of Gallic acid prepared with the suitable blend of Carbapol, Eudragit L100, Guar gum, sodium alginate, calcium chloride and sodium bicarbonate, demonstrates satisfactory release, floating and mucoadhesive properties. The developed floating mucoadhesive microsphere also show good physicochemical properties, antioxidant property, anti-miccrobial property. Drug release from the formulation followed Higuchi model and the mechanism of drug release was diffusion controlled.

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