ORIGINAL ARTICLE

In Vitro and *In Vivo* Evaluation of Mucoadhesive Microspheres for Treatment of *Helicobacter pylori* and Its Associated Diseases

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Abstract

Aim: The aim of the research work was formulation and systemically characterization of *in vitro* and *in vivo* activity of mucoadhesive microspheres of amoxicillin and famotidine for the effective use in the treatment of gastric and duodenal ulcers, which are associated with *Helicobacter pylori*. Such that the dual therapy gives better *H. pylori* eradication than single-drug therapy. The presence of two drugs in the same system will lead to improved efficacy of the system and increased patient compliance. **Materials and Methods:** Drugs containing mucoadhesive microspheres were prepared by an emulsion-solvent evaporation method using carbopol-934P as mucoadhesive polymer and ethyl cellulose as carrier polymer. The preliminary studies included the formulation of 27 batches of each drug using 3^3 factorial design. **Optimization:** A 3^3 factorial design was used to study the effect of independent variables, drug-to-polymer ratio (amoxicillin/famotidine-ethyl cellulose-carbopol-934P) (X_1), concentration of emulsifying agent (X_2), and stirring speed (X_3) on dependent variables, drug entrapment efficiency (Y_1), and particle size (Y_2). Further, the *in vitro* mucoadhesion test was carried out for the mucoadhesion percentage and finally the *in vivo* studies (Bacterial clearance study, *in vivo* mucoadhesion and *in vivo* ulcer index studies) were carried out. **Results and Discussion:** Among the formulated batches, the batch A27 exhibited the best percent of mucoadhesion 66% after 10 h and F24 showed 74% of mucoadhesion after 10 h. Both batches were evaluated for *in vivo* performance. In the bacterial clearance studies, the mean bacterial count (log colony forming units) after oral administration of drug-loaded microspheres was found to be 3.72 ± 0.58. The drug-loaded microspheres formulation exhibited better clearance from infection than plain drugs solution at the same doses. Drug microspheres formulation was found to be effective in the treatment of *H. pylori* infections effectively, and in *in vivo* mucoadhesion studies, the developed system was well taken up and processed by the cells of gastric mucosa of the stomach.

Key words: Factorial design, gastroretentive, *Helicobacter pylori*, optimization

INTRODUCTION

Oral ingestion is the most convenient and commonly used method of drug delivery. More than 50% of drug delivery systems available in the market are oral drug delivery systems. These systems have the obvious advantages of ease of administration and patient acceptance. Attempts to develop a single-dose therapy for the whole duration of treatment have focused attention on controlled or sustained release drug delivery systems.

Under certain circumstances, prolonging the gastric retention of a delivery system is desirable for achieving greater therapeutic benefit of the drug substances. For example, drugs that are absorbed in the proximal part of the gastrointestinal tract[1] and the drugs that are less soluble or degraded by the alkaline pH may benefit

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from prolonged gastric retention. In addition, for local and sustained drug delivery to the stomach and the proximal small intestine to treat certain conditions, prolonging gastric retention of the therapeutic moiety may offer numerous advantages including improved bioavailability, therapeutic efficacy, and possible reduction of the dose size. It has been suggested that prolonged local availability of antibacterial agents may augment their effectiveness in treating *Helicobacter pylori*-related peptic ulcers.

Gastroretentive systems can remain in the gastric region for several hours and hence significantly prolong the gastric residence time (GRT) of drugs. Prolonged gastric retention improves bioavailability, reduces drug waste, and improves solubility for drugs that are less soluble in a high-pH environment. It has applications also for local drug delivery to the stomach and proximal small intestine. Gastro retention helps provide a better availability of new products with new therapeutic possibilities and substantial benefits for patients. One of the most feasible approaches for achieving a prolonged and predictable drug delivery profile in the gastrointestinal tract is to control the GRT. Dosage forms with a prolonged GRT, i.e., gastroretentive dosage forms (GRDFs), will provide with new and important therapeutic options.

Gastric retention provides advantages such as the delivery of drugs with narrow absorption windows in the small intestinal region. Furthermore, a longer residence time in the stomach could be advantageous for local action in the upper part of the small intestine, for example, treatment of peptic ulcer disease.

GRDFs greatly improve the pharmacotherapy of the stomach through local drug release drug concentrations at the gastric mucosa (eradicating *H. pylori* from the submucosal tissue of the stomach), making it possible to treat stomach and duodenal ulcers, gastritis, and esophagitis.

Microspheres carrier systems made from the naturally occurring biodegradable polymers have attracted considerable attention for several years in sustained drug delivery. However, the success of these microspheres is limited due to their short residence time at the site of absorption. It would, therefore, be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membranes and specific targeting to the site. This can be achieved by coupling bioadhesion characteristics to microspheres and developing characteristics to microspheres and developing mucoadhesive microspheres.[2]

H. pylori is a major gastric pathogen in the worldwide distribution; these are spiral-shaped bacteria found in the stomach, which (along with acid secretion) destroy stomach along with the duodenal tissue, resulting in inflammation and peptic ulcers. The method by which *H. pylori* may cause gastroduodenal disease and contribute to gastric carcinogenesis is still hypothetical.

Several treatment regimens are emerging for *H. pylori* infection from the early 1990s, when monotherapy was first recommended. Even though *H. pylori* is highly sensitive to most of the antibiotics, its elimination is not easy even with the best currently available therapy.[3] Antimicrobial therapy for this infection is a complex issue, and the following drugs are currently used in combination regimens: Proton-pump inhibitors and/or bismuth, metronidazole, clarithromycin, and amoxicillin.[4,5] Although optimal first-line treatment is associated with high cure rates, the rising prevalence of resistance to the antibiotic component of current eradication regimens increasingly threatens to compromise the efficacy of these regimens. Strains resistant to metronidazole[6] and clarithromycin[7] have been well documented while resistance to amoxicillin[8] and tetracycline was mainly reported in Asia.[9] Therapeutic regimens directed against *H. pylori* infection will continue to evolve. What is required is a simpler and more efficacious strategy for the treatment of *H. pylori* infection. *H. pylori* is susceptible to many antibiotics *in vitro*, but has proved difficult to eradicate (to root out) *in vivo*.

Amoxicillin (-amino-hydroxybenzylpenicillin) is a semi-synthetic, orally absorbed, broad-spectrum antibiotic. It is now widely used in the standard eradication treatment of gastric and duodenal ulcers, which are associated with *H. pylori* infection combined with a second antibiotic and an acid-suppressing agent.[10-12] These triple therapies have proved to be effective in clinical application. However, some other reports and clinical trials indicate that the therapies cannot bring out complete eradication of *H. pylori* and suggest that the therapeutic effect needs more investigation.[13,14] One reason for the incomplete eradication of *H. pylori* is probably due to the short residence time of dosage form in the stomach so that effective antimicrobial concentration cannot be achieved in a gastric mucous layer or epithelial cell surfaces where *H. pylori* exists.[15,16] The other may be the degradation of amoxicillin in gastric acid.[17,18] Therefore, some researchers had prepared and reported new amoxicillin formulations such as float tablets, mucoadhesive tablets, and pH-sensitive excipients composition microspheres, which were able to reside in the gastrointestinal tract for an extended period for a more effective *H. pylori* eradication.[19,20]

Famotidine is a histamine H_2-receptor antagonist. It is mostly prescribed for gastric ulcers, duodenal ulcers, also for gastroesophageal reflux diseases. With low bioavailability (40-45%) and short biological half-life (2.5-4.0 h), it favors the development of a sustained release formulation. It has been reported that the oral treatment of gastric disorders with an H2 receptor antagonist such as famotidine promotes local delivery of these drugs to the receptor of parietal cell wall. Local delivery also increases the stomach wall receptor site bioavailability and increases the efficacy of drugs to reduce acid secretion. Hence, this principle may be applied for improving systemic as well as local delivery of famotidine, which would efficiently reduce gastric acid secretion.[21,22]

Therefore, it is expected that if local delivery of antimicrobial agents from the gastric lumen into the mucous layer can be achieved, the *H. pylori* eradication rate will be increased.[23]

In the context of the above principles, a strong need was felt to develop a dosage form that delivered amoxicillin and famotidine in the stomach and would increase the efficiency of the drug, providing sustained action. Thus, an attempt was made in the present investigation to use carbopol-934P as a mucoadhesive polymer and ethyl cellulose as a carrier polymer and prepare mucoadhesive microspheres. The microspheres were characterized by *in vitro* and *in vivo* tests and factorial design was used to optimize the variables.

MATERIALS AND METHODS

Drug-loaded mucoadhesive microspheres were prepared by emulsion solvent evaporation method using Carbopol 934 and ethyl cellulose as polymers. In the first step, ethyl cellulose was dissolved in 200 ml of ethanol and then drug and polymer were dispersed in the solution of ethyl cellulose under stirring. The preliminary trial batches were prepared and optimized using 3^3 factorial design earlier by varying the drug-to-polymer-to-polymer (amoxicillin/famotidine-ethyl cellulose-carbopol-934P) ratio in the range of 1:3:1% to 1:3:3%. The final mixture was extruded through a syringe (gauge No. 20) in 500 ml of liquid paraffin (mixture of heavy and light, 1:1 ratio) containing Span 80 and stirring was carried out using a propeller stirrer (Remi, Mumbai, India) at 1000 rpm. The stirring was done for 3 h. In preliminary trial batches, the amount of emulsifying agent (1-3%), the drug:polymer concentration 1:3:1% to 1:3:3%, and stirring speed (500-1000 rpm) were varied.

For optimization, factorial design approach was used, in total, 27 formulations were prepared, the drug-to-polymer-to-polymer ratio, concentration of emulsifying agent, and stirring speed were varied, and all other parameters were kept same. The microspheres prepared were filtered and washed several times with petroleum ether (80:20) to remove traces of oil. The microspheres were then dried at room temperature (25°C and 60% RH) for 24 h. Formulation tables are given in Tables 1 and 2.

EVALUATION

In vitro mucoadhesion test for microspheres

The *in vitro* mucoadhesion test as reported by Lehr *et al.* was used for the evaluation of percent mucoadhesion. A 1 cm × 1 cm piece of rat stomach mucosa was tied onto a glass slide using thread. Microspheres were spread (~50) onto the wet rinsed tissue specimen and the prepared slide was hung onto one of the groves of a USP tablet disintegrating test apparatus with continuous oxygen supply. The disintegrating test apparatus was used where the tissue specimen was given regular up and down movements in the beaker of the disintegration apparatus, containing gastric fluid (pH 1.2), for 10 h, the number of microspheres still adhering onto the tissue was counted [Figure 1].

In vitro drug release study

The drug release study for amoxicillin microspheres and famotidine microspheres was carried out using USP XXIV basket apparatus (Electrolab, TDT-06T, India) at 37°C ± 0.5°C and at 100 rpm using 900 ml of phosphate buffer (pH 7.8) and (0.1 N HCl) as a dissolution medium (n = 5) as per USP XXVI dissolution test prescribed for tablets. Microspheres with the weight equal to 100 mg of drug were used for the test. A volume of 5 ml sample from solution was taken at predetermined time intervals and was filtered through a 0.45-µm membrane filter, properly diluted, and analyzed spectrophotometrically. An equal amount of fresh dissolution medium was added immediately after taking the test sample. The dissolution rate of drug dissolved at various intervals was calculated [Table 3].

In vivo studies

The *in vivo* studies of mucoadhesive microspheres containing amoxicillin and famotidine were performed by three different studies:

- Bacterial clearance STUDIES (for amoxicillin)
- *In vivo* mucoadhesion studies (for estimation of mucoadhesion)
- *In vivo* ulcer index studies (for effect of famotidine).

Bacterial clearance study

The experimental protocol 1227/ac/08/CPCSEA was duly approved by the Institutional Ethical Committee of Smriti College of Pharmaceutical Education, Indore, Madhya Pradesh, India. The studies were carried out in accordance with the Council for Purpose and Supervision of Experiment

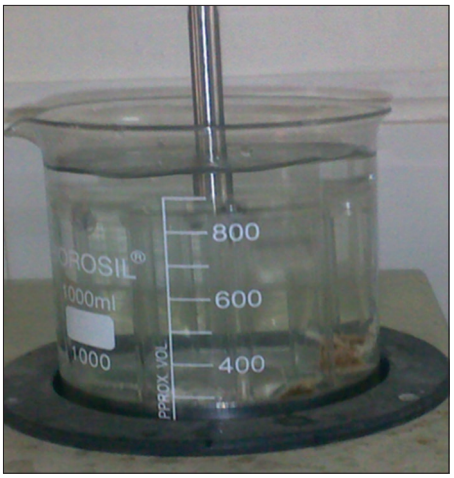

Figure 1: *In vitro* wash-off test assembly

Table 1: Factorial design batches (amoxicillin)

Batch code	Variables in coded form				
	X_1	X_2	X_3	Y_1	Y_2
	Polymer concentration	Emulsifying agent	Stirring speed	Drug entrapment (%)	Particle size (μm)
A1	−1.00	−1.00	−1.00	28	35
A2	0.00	−1.00	−1.00	48	38
A3	1.00	−1.00	−1.00	63	54
A4	−1.00	0.00	−1.00	31	39
A5	0.00	0.00	−1.00	39	42
A6	1.00	0.00	−1.00	51	58
A7	−1.00	1.00	−1.00	36	41
A8	0.00	1.00	−1.00	48	52
A9	1.00	1.00	−1.00	56	57
A10	−1.00	−1.00	0.00	35	34
A11	0.00	−1.00	0.00	51	48
A12	1.00	−1.00	0.00	65	63
A13	−1.00	0.00	0.00	41	50
A14	0.00	0.00	0.00	56	51
A15	1.00	0.00	0.00	61	47
A16	−1.00	1.00	0.00	45	54
A17	0.00	1.00	0.00	62	62
A18	1.00	1.00	0.00	61	58
A19	−1.00	−1.00	0.00	52	45
A20	0.00	−1.00	1.00	51	47
A21	1.00	−1.00	1.00	54	51
A22	−1.00	0.00	1.00	54	57
A23	0.00	0.00	1.00	57	59
A24	1.00	0.00	1.00	64	43
A25	−1.00	1.00	1.00	54	47
A26	0.00	1.00	1.00	58	64
A27	1.00	1.00	1.00	66	99

Coded values	Actual values		
	X_1 (mg)	X_2 (%)	X_3 (rpm)
Low (−1)	100	1	800
Medium (0)	200	2	1000
High (1)	300	3	1200

X_1: Polymer concentration, X_2: Emulsifying agent, X_3: Stirring speed. Further, Batch No: A12, A24, and A27 were selected for further study on the basis of good drug entrapment efficiency

on Animals, the Ministry of Social Justice and Empowerment Government of India.

Animal required: Male Sprague-Dawley rats.

Weight: 200-250 g.

Sex: Male, 6-8 weeks old.

Total number of rats required: 30.

H. pylori strain obtained from Department of Microbiology.

The method used for *in vivo* study for *H. pylori* is known as Qian's method.

Preparation of pathogenic H. pylori culture

Gastric biopsy specimens were collected. Each taken biopsy was homogenized with two drops of *Brucella* broth in sterile glass homogenizer and inoculated on *Brucella* chocolate agar plate. The plates were incubated at 37°C under microaerophilic conditions in a candle jar for 72 h. Subculturing of *H. pylori* was done in *Brucella* broth medium to get pure bacteria. The detection of *H. pylori* was assessed microscopically and by biochemical tests including catalase and oxidase tests.

Table 2: Factorial design batches of famotidine

Batch code	Variables in coded form				
	X_1	X_2	X_3	Y_1	Y_2
	Polymer concentration	Emulsifying agent	Stirring speed	Drug entrapment (%)	Particle size (μm)
F1	−1.00	−1.00	−1.00	28	35
F2	0.00	−1.00	−1.00	48	38
F3	1.00	−1.00	−1.00	63	54
F4	−1.00	0.00	−1.00	31	39
F5	0.00	0.00	−1.00	39	42
F6	1.00	0.00	−1.00	51	58
F7	−1.00	1.00	−1.00	36	41
F8	0.00	1.00	−1.00	48	52
F9	1.00	1.00	−1.00	56	57
F10	−1.00	−1.00	0.00	35	34
F11	0.00	−1.00	0.00	51	48
F12	1.00	−1.00	0.00	56	63
F13	−1.00	0.00	0.00	41	50
F14	0.00	0.00	0.00	65	51
F15	1.00	0.00	0.00	61	47
F16	−1.00	1.00	0.00	45	54
F17	0.00	1.00	0.00	62	62
F18	1.00	1.00	0.00	61	58
F19	−1.00	−1.00	0.00	52	45
F20	0.00	−1.00	1.00	51	47
F21	1.00	−1.00	1.00	54	51
F22	−1.00	0.00	1.00	54	57
F23	0.00	0.00	1.00	57	59
F24	1.00	0.00	1.00	64	43
F25	−1.00	1.00	1.00	69	47
F26	0.00	1.00	1.00	58	64
F27	1.00	1.00	1.00	54	76

Coded values	Actual values		
	X_1 (mg)	X_2 (%)	X_3 (rpm)
Low (−1)	100	1	800
Medium (0)	200	2	1000
High (1)	300	3	1200

X_1: Polymer concentration, X_2: Emulsifying agent, X_3: Stirring speed. Batch No: F14, F24, and F25 were selected for further study on the basis of good drug entrapment efficiency

Inoculation of H. pylori and administration of formulations to rat

All 6-8 weeks old male Sprague-Dawley rats were fasted overnight before inoculation. A volume of 1 ml broth containing about 1010 colony forming units (CFUs) of *H. pylori* per mL was inoculated into the stomach of each rat via a gastric cannula. Bacterial colonization was assessed 4 weeks post inoculation in a given rats by bacterial culture and histology of stomach tissue.

The control group received only physiological saline. Formulations were given to each group once daily for 3 days. Table 4 shows the study design.

One day after administration of the final dose, the rats were sacrificed, their stomachs were removed and subjected to the following tests.

Histopathological examination of rat stomach: histopathological examination was done for stomach specimens reserved in

10% formalin which were ranked according to the intensity of *H. pylori* colonization as follows: Severe infection, moderate infection, mild infection, and free from infection.

Clearance of *H. pylori* from rat stomach: Each stomach was homogenized with *Brucella* broth (3 mL/stomach) from which serial dilutions were placed on *Brucella* chocolate agar medium (Difco). The plates were incubated at 37°C in a microaerophilic environment for 3-4 days.

The viable cell count for each gastric wall was calculated by counting the number of colonies on the agar plates. The logarithm of CFUs per gastric wall was expressed as % bacterial recovery. The clearance rate (%) was also calculated by the number of rats cleared from infection per total number of rats used in each group.

In vivo mucoadhesion study

Fluorescence isothiocyanate (FITC) was used as a dye to be incorporated in the system. Sprague-Dawley rats (200-250 g) were fasted for 24 h before experiment but were allowed free access of water. Plain dye (FITC) solution, FITC labeled microspheres were filled in the capsule was administrated to rats using a gastric sonde. After 2 h post administration, the rats were sacrificed and stomach was removed and washed with SGF (pH 1.2) to recover the remaining microspheres.

Table 3: *In vitro* drug release profile

Time	% cumulative drug release (pH 1.2) A27	% cumulative drug release (pH 1.2) F25
0 min	0	0
30 min	7.96	10.3
1 h	15.91	13.42
2 h	21.42	18.25
3 h	26.47	25.01
4 h	38.13	33.76
5 h	51.02	49.00
6 h	58.43	57.60
7 h	69.17	68.13
8 h	82.48	83.04
9 h	88.14	87.68
10 h	90.02	92.03

Microtomy was performed and micro thin sections on the slides were observed under fluorescent microscope (OLYMPUS BX60).

A bright yellowish green fluorescent was observed in the cross section of the stomach.

In vivo ulcer index studies

Albino rats of both sexes (age: 10-12 weeks and weighing 200-250 g) were used in all the experiments. Each group of the treated animals contained at least three rats and was housed in standard cages. Disease control (distilled water-10 ml/kg), famotidine marketed suspension (40 mg/10 ml/kg), and famotidine mucoadhesive microspheres (40 mg/10 ml/kg) were arranged in groups 1, 2, and 3, respectively. Distilled water, drugs, and formulation were given orally, and 30 min later, aspirin (200 mg/kg-per oral) was administrated to all the groups.

8 h later, the animals were killed by decapitation. The stomachs were removed, opened along the great curvature, and washed with the tap water to remove the gastric contents, then examined under a dissecting microscope with a square-grid eyepiece to assess the formation of ulcers. For each stomach, ulcerated and total areas were measured as mm^2. The ulcer indexes for each stomach were calculated using the following formula:

Ulcer index=[Ulcerated area/Total stomach area]*100

RESULTS AND DISCUSSION

Optimization of amoxicillin-loaded microspheres

In the development of mucoadhesive microspheres of amoxicillin and famotidine, a 3^3 full factorial design was employed. Hence, a response surface design model with 3 factors, 3 levels, and 27 runs was selected for the optimization study. The dependent variables obtained at various levels of the 3 independent variables (X_1, X_2, and X_3) were subjected to multiple regression to yield a second-order polynomial equation obtained coefficient. The polynomial equation generated by this experimental design (using Design Expert 7.1.6) was as follows:

$$Y= b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1X_1 + b_{22}X_2X_2 + b_{33}X_3$$

Table 4: Details of study design

Type of study	Animal type	Study design groups	Number of animals required
Histopathological studies, % bacterial recovery and % clearance rate studies	Albino rats	Group 1 (controlled)	6
		Group 2 (plain drugs solution)	6
		Group 3 (drug-loaded microspheres)	6
Fluorescent microscopy	Albino rats	Group 1 (plain dye solution)	2
		Group 2 (dye-loaded microspheres)	2

Where, Y is the dependent variable; b_0 is the intercept; b_1 to b_{33} are the regression coefficients; and X_1, X_2, and X_3 are the independent variables.

The responses were analyzed using ANOVA using Design-Expert version 7. A mathematical equation was generated for each response parameter. Using mathematical models, significance was tested. Response surface plots were generated for each response to study the behavior of the system. Response surface graphs for amoxicillin are shown in Figures 2-5.

Response surface graphs for famotidine are shown in Figures 6-9.

Response surface plots were plotted for every response to check the behavior of the system. The responses were analyzed using ANOVA. A mathematical equation was generated for each response parameter. The mathematical models were tested for significance. Among the 3 independent variables, $P \geq 0.05$, indicating that this variable was insignificant in the prediction of drug entrapment and particle size [Table 5].

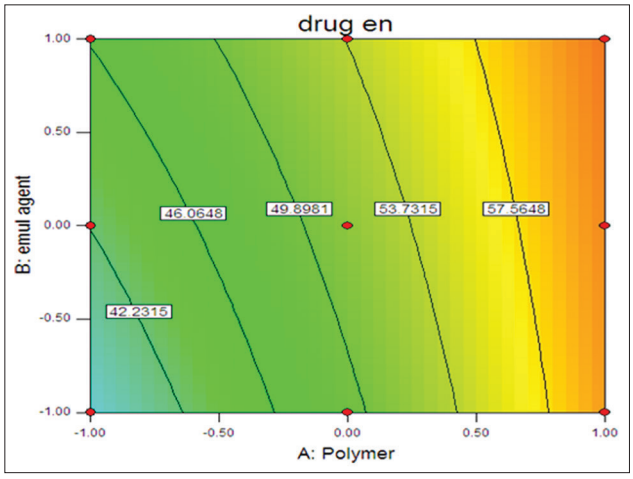

Figure 2: Contour plot of drug entrapment

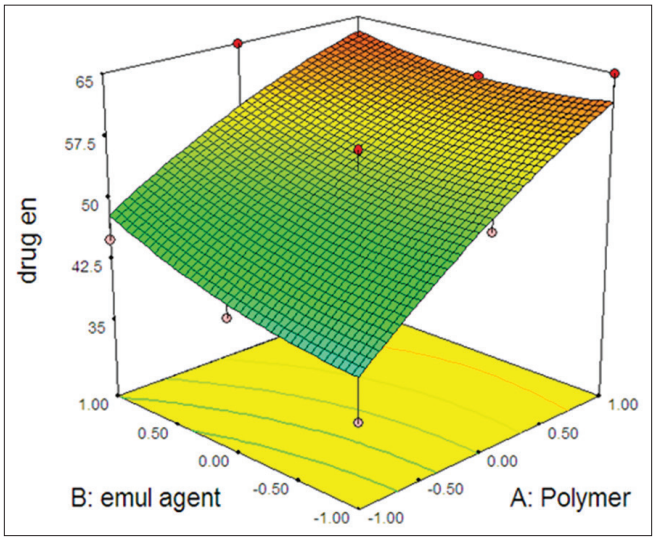

Figure 3: Three-dimensional surface plot for drug entrapment

In vitro mucoadhesion

The *in vitro* mucoadhesiveness test resulted in the percent of mucoadhesion of microspheres remaining on the stomach mucosa [Table 6]. The test was carried out for three best batches among the factorial design batches based on good entrapment efficiency and the batch A27 and F24 showed that even after 10 h, 66% and 74% microspheres were adhered to the gastric mucous layer. The mucoadhesive microspheres were spherical and free flowing.

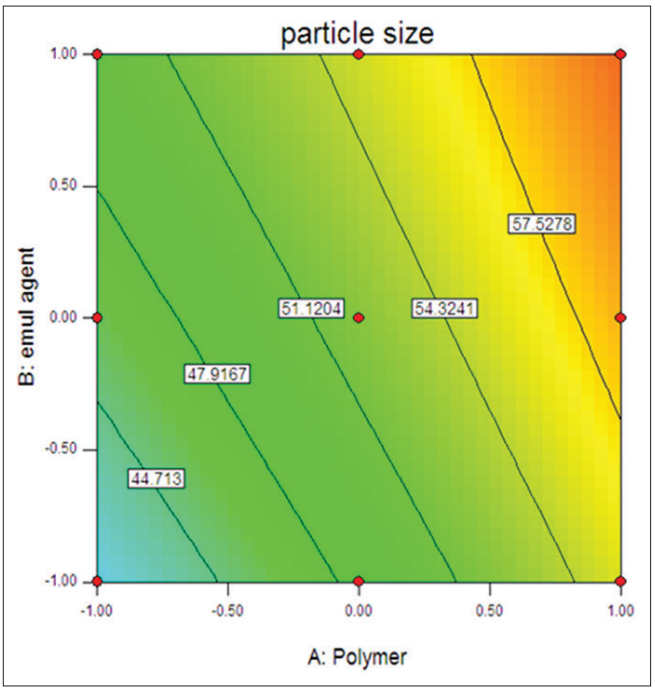

Figure 4: Contour plot of particle size

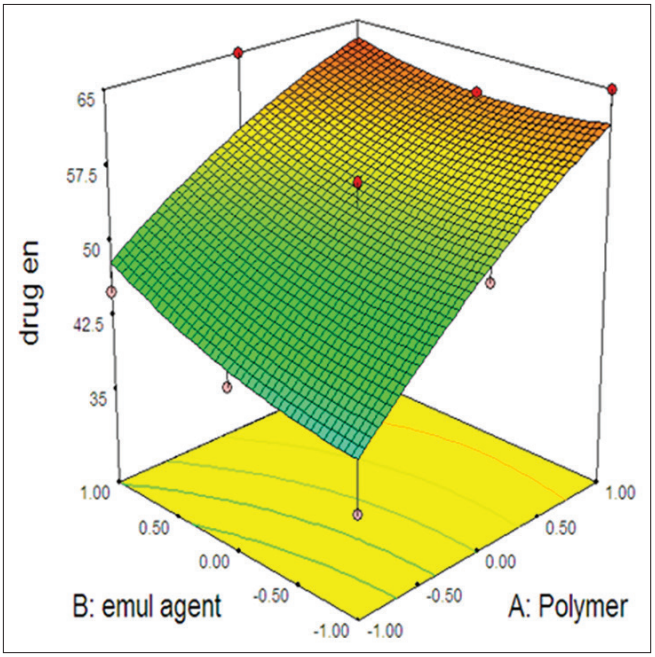

Figure 5: Three-dimensional surface plot for particle size

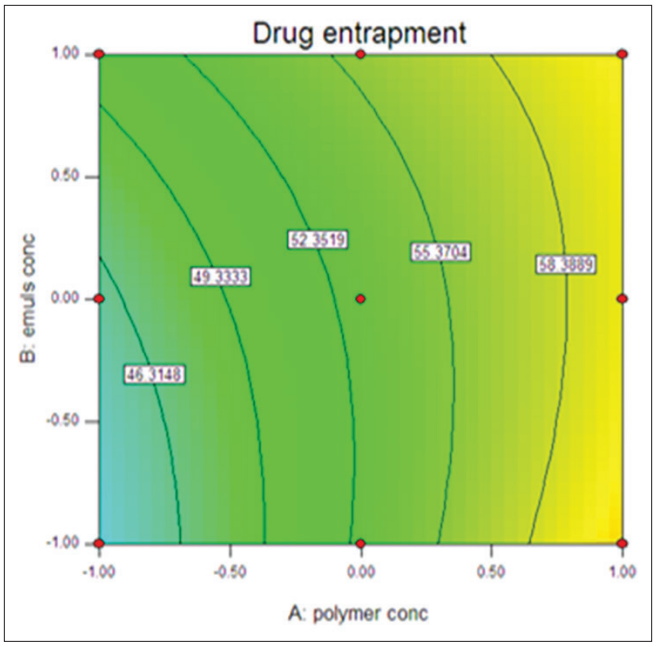

Figure 6: Contour plot of drug entrapment

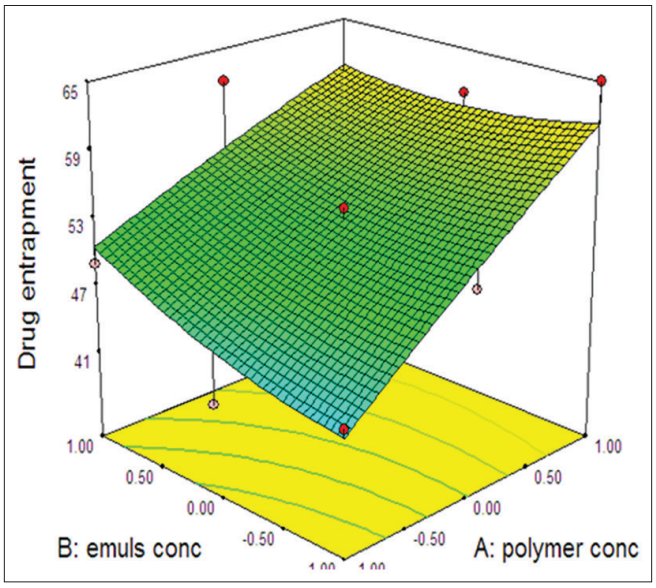

Figure 7: Three-dimensional surface plot of drug entrapment

In vitro drug release studies

A sustained drug release was obtained for more than 10 h [Figure 10].

In vivo studies

Bacterial clearance count

The method described by Ishak *et al.* (2007) was used to assess the *in vivo* usefulness of amoxicillin microspheres for eradication of *H. pylori*. Infecting the animal model, i.e., male albino rats infected with *H. pylori* was used for *in vivo* study. The advantages of this method are that the errors, which

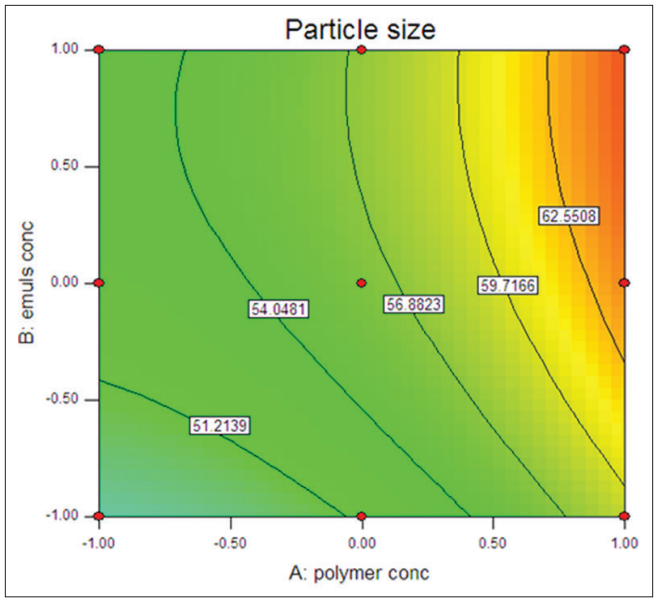

Figure 8: Contour plot of particle size

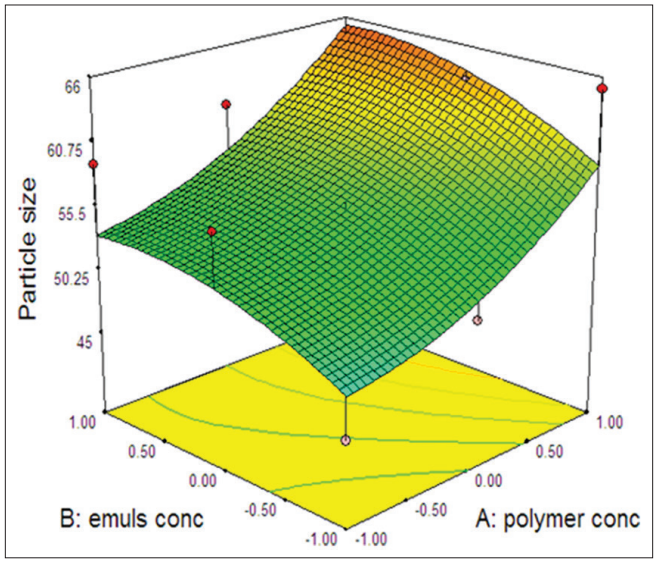

Figure 9: Three-dimensional surface plot of particle size

may occur due to variation in sample site, are avoided as the whole of the stomach is used to determine the bacterial cell count. Albino rats were chosen for the study because they offer several advantages as animal model for oral absorption studies are co-operative, convenient to handle, relatively cheap, readily available, and easy to be housed. The % bacterial recovery and % clearance rate studied of *H. pylori* infections after administration of formulations bearing amoxicillin under fed conditions are presented in Table 7.

The control group of rat received only physiological saline. The mean bacterial count (log CFU) was found to be 9.64 ± 0.35. The mean bacterial count (log CFU) after oral administration of plain drugs solution (amoxicillin) was found to be 5.83 ± 0.23, which is due to unavailability of 100% drugs and short residence time of drugs solution in the stomach and the low concentration

Table 5: Results of ANOVA for dependent variables (amoxicillin)

Response of variables	df	SS	MS	*F*	R^2
For drug entrapment					
Regression	9	2639.38	293.5	14.02	0.8813
Residual	17	355.46	20.91		
Total	26	2994.74			
For particle size					
Regression	9	1182.92	131.44	3.61	0.8898
Residual	17	618.94	36.41		
Total	26	1801.85			

Table 6: Results of ANOVA for dependent variables (famotidine)

Response of variables	df	SS	MS	*F*	R^2
For drug entrapment					
Regression	9	1463.31	162.59	8.44	0.8170
Residual	17	327.66	19.27		
Total	26	1790.96			
For particle size					
Regression	9	980.03	108.89	3.37	0.8250
Residual	17	549.60	32.33		
Total	26	1529.63			

Table 7: Percent mucoadhesion of selected batches

Batch number	1 h (%)	5 h (%)	10 h (%)
Amoxicillin-loaded batches			
A12	69	54	48
A24	81	72	62
A27	79	68	66
Famotidine-loaded batches			
F14	78	67	60
F24	81	72	66
F25	84	78	74

of drugs reaching the *H. pylori* under the gastric mucus layer. The mean bacterial count (log CFU) after oral administration of drug-loaded microspheres was found to be 3.72 ± 0.58. The drug-loaded microspheres formulation exhibited better clearance from infection than plain drugs solution at the same doses. Drug microspheres formulation was found to be effective in the treatment of *H. pylori* infections effectively [Table 8].

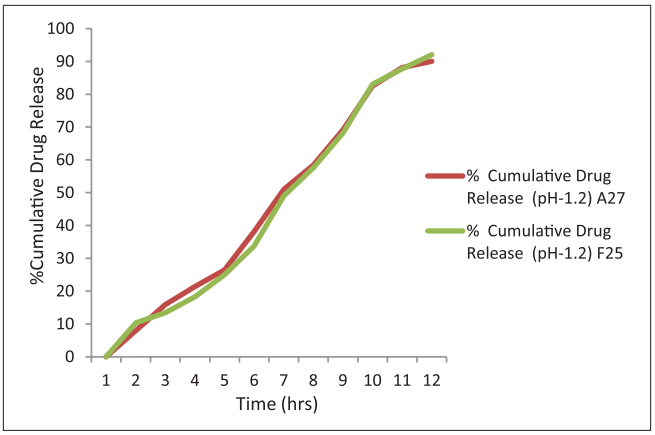

Figure 10: *In vitro* drug release profile of formulation A27 and F25

From the histopathological examination, it is obvious that group of rats receiving plain drugs solution at dose amoxicillin 30 mg/kg showed moderate infection with a large population of *H. pylori.*

Group of rats receiving microspheres formulation at the same doses showed mild infection with a large population of *H. pylori*. From the histopathological examination, it is obvious that groups receiving drugs in the form of the formulation at doses amoxicillin 30 mg/kg were better than the corresponding solution form at same doses, to eradicate the *H. pylori* infection. This means that drugs formulation provided 2-3 times greater anti-*H. pylori* activity than plain drugs solution, respectively, due to their mucoadhesive nature and increased residence time in the stomach.

In vivo mucoadhesion study

The mucoadhesive behavior of the microspheres was determined by determining the amount of microspheres, which were obtained after 2 h of incubation of films pieces in the stomach and intestine.

The muco-adhesivity of the microspheres also is partly responsible for the prolongation of the residence time of the system in the mucus layers. This leads to more absorption of drugs. This muco-adhesivity was also confirmed *in vivo* using FITC dye with the formulation. This study is also confirmed the muco-adherence potential of the formulation.

This clearly indicates that the developed system was well taken up and processed by the cells of gastric mucosa of the stomach. This shows that the system could attach the mucosal gel layer where *H. pylori* resides [Figures 11 and 12].

In vivo ulcer index studies

The disease-controlled model in rat after the oral administration of aspirin establishes a stable ulcer for at least 2 days. This permits the characterization of the *in vivo* deposition of the

Table 8: Effect of different preparation on clearance rate and % bacterial recovery

Preparations	Clearance rate (%)	Bacterial recovery (log of CFU per gastric wall) %
Control	0 (0.6)	9.64±0.35
Plain drugs solution (amoxicillin)	33.33 (2.6)	5.83±0.23
Drug-loaded mucoadhesive microspheres (A-27)	66.67 (4.6)	3.72±0.58

N=6; ±SD. SD: Standard deviation, CFU: Colony forming units

particulate carrier system under the influence of gastritis symptoms. After inducing the gastric ulcer, stomach was opened to get visual evidence of the ulceration and to characterize the differences to healthy tissue [Figure 13]. In the histological analyses, strong damages of the gastric tissue were observed. In addition, it was observed that the stomach wet weight/body ratio increased compared to the healthy control group, which has been known as an indicator for inflammation.

The behavior of the proposed microsphere system was examined with respect to reduction in ulcer index and accumulation in the ulcerated gastric tissue after oral administration. Mucoadhesive microsphere dispersion showed a significant decrease in ulcer index (0.46 ± 0.011) when compared with the control group (3.61 ± 0.14) and famotidine suspension-treated animal (0.66 ± 0.035). Qualitatively, an increased adherence of the mucoadhesive microspheres was obtained in ulcerated tissue. A size-dependent particle deposition in the gastrointestinal tract of healthy subjects as well as mucoadhesion has been reported in literature depending on the particle surface properties (Hasani *et al*. 2009).

In the present work, famotidine microspheres surface properties were modified with mucoadhesive polymers. Ulcer index of mucoadhesive microspheres dispersion-treated animal was dramatically reduced compared to famotidine suspension-treated animal. It was noticed that mucoadhesive microspheres with the smallest diameter led to the highest adhesion compared to the coarse particle of famotidine suspension. This could be attributed to the strong mucus production in the gastrointestinal tract, especially in the stomach, which favored the particle adhesion to the mucus. Mucoadhesive microspheres can better attach to mucus layer due to their small mass.

CONCLUSION

The mucoadhesive microspheres containing amoxicillin and famotidine were developed using a 3^3 factorial design and showed a high percentage of mucoadhesion, drug entrapment efficiency, and exhibited a sustained release property. The effect

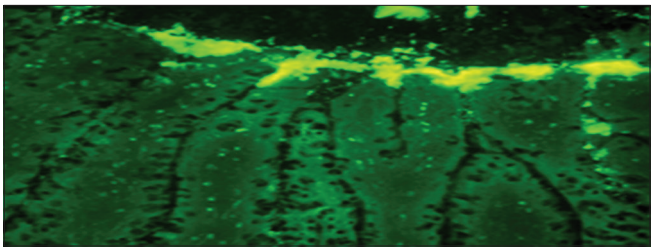

Figure 11: Fluorescent photographs of plain fluorescence isothiocyanate solution

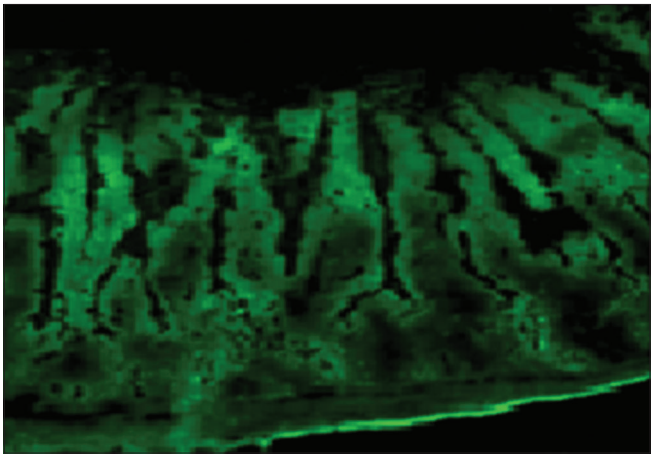

Figure 12: Fluorescent photographs of fluorescence isothiocyanate-labeled microspheres in gastric tissue (after 2 h)

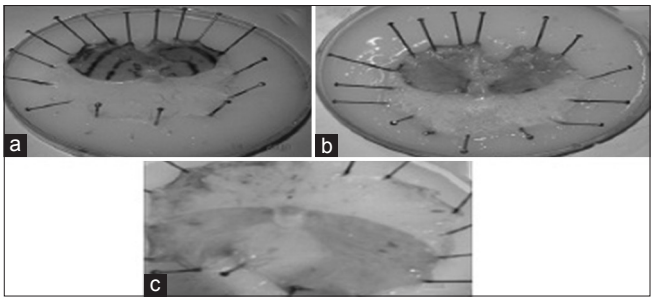

Figure 13: (a) Ulcer-induced rat stomach. (b) Famotidine suspension-treated. (c) Mucoadhesive microsphere-treated

of drug-to-polymer-to-polymer ratio, emulsifying agent, and stirring speed significantly influence on drug entrapment and particle size. The formulation showed more effective *H. pylori* activity of mucoadhesive amoxicillin microspheres compared to amoxicillin and famotidine mucoadhesive microsphere dispersion showed a significant decrease in ulcer index when compared with the control group and famotidine suspension-treated animal, which might indicate a potential use of mucoadhesive microspheres in treating *H. pylori* infection.

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