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Formulation And Evaluation pH Stimuli Sensitive Multiparticulate Delivery System Of Clarithromycin For The Treatment Of Helicobacter pylori

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Abstract: pH stimuli sensitive microspheres of clarithromycin were prepared on the principle of cation induced gelification, whereas, sodium alginate and pectin blend were cross linked with calcium chloride and investigated with regard to drug targeting specificity in the gastric mucosa. The microspheres were found to be discrete, spherical, free flowing and of monolithic matrix type. The yields of microspheres were found to varied 65±2 to 76±3%. Prepared microspheres have shown good mucoadhesive efficacy and anti microbial activity against isolated *Helicobacter pylori* strain. The following repeated oral administration of the formulation to *Helicobacter pylori* infected Mongolian gerbils was examined by polymerase chain reaction technique. It was found that the coated formulation was more effective than clarithromycin suspension. The results further substantiated that pH sensitive microspheres were found to adhere to the gastric or intestinal mucosa with high affinity might make contribution for complete eradication of *Helicobacter pylori.*
Keywords: Therapeutic efficacy, Polymerase chain reaction, Gastric mucosa, Lymphoma, *Helicobacter pylori* gene, Primers, Biphasic release, Means residence time.

Introduction

The whole healthcare profession strive towards improving health outcomes by targeted delivery of anti-infectious agents for the cure of microbial infection in the gastrointestinal tract. *Helicobacter pylori* (*H.pylori)* is a small, spiral-shaped, microaerophilic, gram negative bacterium and causative pathogen of peptic ulcer, chronic gastritis, gastric mucosa associated lymphoid tissue lymphoma, and in the development of gastric cancer [1]. However, the immune system produces antibodies against the bacterium virtually in all infected patients; immonuglobulin activity is largely inhibited by the gastric acid. Moreover, due to its motility, the bacterium may be inaccessible to direct contact with immune cells [2]. The treatment of *H. pylori* remains a challenging proposition; however, development of antibiotic resistance in *H. pylori* and degradation of antimicrobial agents by gastric acid are recognised as a significant contributing factor in *H. pylori* treatment failure [3].The residence time of antimicrobial agents in the stomach is so short that effective antimicrobial concentrations cannot be achieved in the gastric mucous layer or epithelial cell surfaces where *H. pylori* exists [4].

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. Better stability and longer residence time will allow more of the antibiotic to penetrate through the gastric mucus layer to act on *H. pylori* [5]. To improve the therapeutic efficacy some researchers had prepared and reported new formulations of the antibacterial agents, such as floating tablets, mucoadhesive tablets, pH sensitive excipients composition mucoadhesive microspheres,

etc., which were able to reside in stomach for an extended period for more effective *H. pylori* eradication [6]. Gastro retentive drug delivery systems like floating and bioadhesive systems have reported to improve therapeutic effects of antimicrobial drugs. The prolonged residence time of delivery system is anticipated to allow more of the antibiotic to penetrate through the gastric mucus layer to eradicate *H. pylori*, thereby improving therapeutic efficacy.

Chun et al. 2005 [7] prepared and evaluated the oral mucoadhesive microspheres of Cl and amoxicillin which exhibited diffusion and dissolution controlled release mechanism respectively. Umamaheswari and Jain [8] reported receptor mediated targeting of lectin conjugated gliadin nanoparticles bearing acetohydroxamic acid. Jain et al 2009 [9] designed mucoadhesive multiparticulate delivery system of Cl for the effective treatment of *Helicobacter pylori* colonization.

Clarithromycin is a macrolide based broad-spectrum antibiotic. It is widely used in a standard eradication treatment of gastric *H. pylori* infection and the antibiotic has highest eradication rate in monotherpy in vivo [10].Oral controlled drug delivery systems based on blend polysaccharides matrix are gaining widespread acceptance in the pharmaceutical industries because of their flexibility to obtain a desirable drug-release profile, cost effectiveness, and broad regulatory acceptance. However the resent advances of the blend polymers take the advantages of their parent polymers and/or create useful new properties. Such a blend matrix can be applied for the release of both hydrophilic, hydrophobic drugs and charged solutes. The aim of the present work was to develop pH sensitive clarithromycin loaded mucoadhesive microparticulate system that would achieve continuous release of the drug in the gastric region and thus be useful for complete termination of the microbial infection at gastric sites.

Material and Methods

Materials

Clarithromycin (Cl) was obtained as a gift sample from Ranbaxy laboratory Ltd., India. Sodium alginate and pectin were purchased from SD Fine Chem Ltd., India. Modified skirrow's medium, brucella broth and fetal calf serum (FCS) were purchased from Himedia, India. Agarose was purchased from FMC BioProducts (Rockland, USA) and Taq DNA polymerase was purchased from Takara Shuzo, Japan. All other reagents were of analytical grade.

Microorganism

The bacterial strain used for the study was isolated with gastric biopsy from the patients suffering with chronic gastritis and peptic ulcer in Institute of Medical Science, Banaras Hindu University, Varanasi, India.

Animals

The animals were housed in a commercial barrier production facility in solid-bottom polycarbonate cages, on hardwood bedding, and covered with stainless steel wire tops and were specific pathogen free (SPF). The animals experienced a 12:12 h light : dark cycle and received HEPA-filtered air at a constant temperature of 21±1°C, with relative humidity controlled at 30 % to 70 % with free access to a commercial gerbil sterilized diet (Hindustan animal feed-India) and UV-treated, filtered, hyper chlorinated water ad libitum. Seven weeks old specific pathogen free male Mongolian gerbils with body mass 65±6 g were used for the present study. The *in vivo* and *ex vivo* studies were performed following the guidelines approved by the Committee for the purpose of control and supervision of experiments on animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. Institutional Animal Ethical Committee of Institute of Medical Science Banaras Hindu University, Varanasi-India , granted permission for the study.

Preparation of microspheres

Microspheres were prepared employing method of Rajaonarivony et al. [11] with slight modifications. The principle involves cation-induced controlled gelification of the polymer. Aqueous blend dispersion of sodium alginate: pectin was prepared in the ratio of 0.5:1.5, 0.75: 1.25, 1.5: 0.5, 1.75: 0.25 and 1: 1 on m/m basis with proper mixing on magnetic stirrer (Table 1). Clarithromycin (0.50 % m/m) was added in polymeric dispersion with continuous stirring for 5 minutes. Calcium chloride (1 mL, 18 mmol L^{-1}) was added into 20 mL of the drug polymer dispersion followed by stirring for 5-30 minutes to complete the curing reaction and to produce spherical and rigid microspheres. The microspheres so prepared were collected by decantation technique, washed repeatedly with deionized water and dried at 45 ^{0}C for 12 hr.

Morphology and particle size

The surface morphology of the microspheres was investigated using scanning electron microscopy ((SEM). The microspheres were coated with gold-palladium under an argon atmosphere using a gold sputter module in a high vacuum evaporator. The coated samples were then randomly scanned and photomicrographs were taken with a scanning electron microscope (Joel 6100, Japan).The particle size of the microspheres was determined by using optical microscop (Labomed CX RIII, Ambala, India).Three hundred dried microspheres were measured for calculating the mean diameter of microspheres.

Drug entrapment and percent

Hundred milligrams of the microparticles were placed in 100 mL of phosphate buffer (pH 7.4) and allowing it to disintegrate completely for 4 hr. The drug concentration in the buffer was analyzed at 353 nm using UV-visible spectrophotometer (Systronics, India) and determined the encapsulation efficiency and drug content [12].

Buoyancy

The buoyancy of microspheres was determined by the reported method with a slight modification [12]. Briefly, the dried microspheres (100 mg) were taken in the test medium (SGF, pH 1.2) was agitated on a magnetic stirrer at 75 rpm and 37 °C. The floating behavior of the microspheres in each test medium was observed.

Measurement of in vitro drug release

The release rate of clarithromycin was determined in a USP XXIII paddle type dissolution apparatus. A weighed quantity of microspheres equivalent to 100 mg of Cl was filled into a hard gelatin capsule (#2) and placed in the paddle of dissolution apparatus. The dissolution medium (900 mL) of SGF (pH 1.2) or phthalate buffer solution (pH 3.4) was used as dissolution medium. The dissolution fluid was maintained at 37±0.5 ^{0}C and rotation speed of 100 rpm. The sample of 5 mL was withdrawn at each 30 min interval and was filtered through 0.25 µm membrane filter. The initial volume of the dissolution fluid was maintained by adding 5 mL of fresh dissolution fluid after each withdrawal. Samples were analyzed at 353 nm. The release data was subjected to model fitting using the PCP Disso 2.0 software, Pune, India. The *in* v*itro* drug release (SGF and phthalate buffer solution) was carried out for marketed uncoated dosage form of Cl (Pylokit-50 mg, tablet) as well following the same procedure.

Coating of microspheres

Having higher encapsulation efficiency, acceptable limits of % buoyancy and % yield batch F_3 was selected for the optimization by coating with ethyl cellulose (Table 2).One hundred milligrams of the microspheres were mixed in 25 mL ethyl cellulose (0.5 to 1.5 % m/V) solution prepared in acetone. The mixture was agitated for 5 min at 400 rpm to complete the process of microspheres coating. Coated microspheres were collected and dried at room temperature up to 24 hours until all solvent was evaporated, leaving a film of ethyl cellulose (EC) coat on the microspheres.

Table 1. Composition and characterization of prepared microspheres

Formulation code	SA:P (m/m)	yield (%)[a]	Mean particle size (µm)[a]	Buoyancy (%)[a]	Encapsulation Efficiency (%) [a]
F_1	0.5:1.5	65 ±2	16 ±1	75±3	72±1.4
F_2	0.75:1.25	75± 2	22 ±1	60±4	78±1.6
F_3	1.5:0.5	76± 3	17±1	86±4	83±1.3
F_4	1.75:0.25	74± 2	15±2	80 ±3	71±1.8
F_5	1.0:1.0	69±3	14 ±2	82±4	79±1.2

SA- sodium alginate, P- pectin , [a] Mean ± SD, n = 3

Table2. Coating and evaluation parameters of the coated F_3 batch

Formulation Code	Ethyl cellulose (% m/V)	DE $_{240\ min}$ (%)	R
F_{13}	0.5	50.8 ±1.3	0.9832
F_{23}	1.0	48.5±1.4	0.9456
F_{33}	1.5	46.4± 1.5	0.9644

DE - dissolution efficiency

Percent mucoadhesion

Albino rats (450-500 g, male) were fasted overnight and dissected immediately after being sacrificed. The stomachs of the rats were removed, cut into pieces 2 cm long and 1 cm wide and rinsed with 2 mL of physiological saline. One hundred milligram of microspheres were scattered uniformly on the surface of the stomach mucosa. The previous method was used for the determination of percent mucoadesion [12].

In vitro growth inhibition studies

Susceptibility of clinical isolates of H. pylori - A total of 100 *H. pylori* isolates were isolated from gastric biopsy specimens of patients with gastritis or peptic ulcer. To suppress the growth of indigenous or exogenous contaminating bacteria, the isolated biopsy sample was grown in brucellla agar, vancomycin (7 mg mL^{-1}), polymyxin B (2 mg mL^{-1}) and amphotericin B (2 mg mL^{-1}) and incubated at 37 ^{0}C for 7 days in microaerophilic atmosphere (5% O_2, 10% CO_2, 85% N_2).The bacteria were identified as *H. pylori* by Gram's staining, colony characteristics, and biochemical tests such as urease and catalase . Quantitative susceptibility was determined in the isolates of *H. pylori* using E-test method [13]. In brief: colonies of the isolated *H. pylori* strain, obtained from a fresh culture were suspended in 5 mL of Mueller-Hinton broth (BHI, Difco , UK) to achieve turbidity equal to the 3 Mac Farland standard. The suspensions were inoculated with sterile swabs onto 150 mm diameter Mueller-Hinton 7% sheep blood agar plates and the agar surfaces were allowed to dry. Three E-test strips were applied to each plate. Plates were incubated at 37ºC up to five days under microaerobic conditions. Inhibitory concentrations were read at the point where the elliptical zone of inhibition intersected the E-test strip.

Percent growth inhibition of H. pylori - *The* method of under taken study based on turbid metric method with slight modification [14]. To study the relative contributions of drug and drug loaded microspheres to growth inhibition, 5 ml of nutrient broth containing *H.pylori* were transferred into sterile test tubes. Plain drug (Cl) and different formulations were taken containing clarithromycin equivalent to 20 µg mL^{-1} which is twice in concentration with respect to MIC (10 µg mL^{-1}) and added to the tubes and all the tubes were incubated at 37°C in a microaerobic atmosphere for 24 hr. Growth of the bacteria was monitored by measuring the optical density (OD) of the broth cultures .

In vivo *H. pylori* eradication studies

Bacterial inoculation and clarithromycin administration - The animals were divided in to five groups containing six animals in each group. They were inoculated orally with 1 mL culture broth after fasting for 24 hours. Each dose contained 10^9 CFU of *H. pylori*. Fourteen days after the infection, clarithromycin suspension (dispersed well in 0.5% m/V of methylcellulose) and clarithromycin loaded microspheres (containing equivalent amount of Cl) were orally administered once a day for 3 consecutive days at a dose of 10 mg kg^{-1}. The drug free microspheres used as control were administered in the same manner.

Formulation efficacy studies- Three days after administration of the final dose, the Mongolian gerbils were killed and the stomachs were removed. Each stomach was homogenized with Brucella broth (3 mL per stomach), and serial dilutions were plated on modified Skirrow's medium. The agar plates were incubated for 4 days at 37 °C under microaerobic conditions in GasPak (BD Diagnostic Systems, Sparks, USA). The viable cell for each stomach was counted by counting the number of colonies on the agar plates [15].

Extraction of DNA for polymerase chain reaction (PCR) - The gastric tissue was collected by centrifugation from 1 mL of homogenate from each gerbil stomach (16,17). The tissue was suspended in 500 µL of TE(tris ethylene diamine tetra acetate, pH 8.0) containing100 $mmol^{-1}$ NaCl and 100 gmL^{-1} egg white lysozyme, and incubated at room temperature for 10 min. Sodium dodecyl sulphate was added at a final concentration of 0.1%, and the mixture was incubated at 60 °C for 10 min. DNA was then extracted in a mixture of phenol: chloroform (1:1), precipitated at 20 °C for 2 h in the presence of 300 $mmol^{-1}$ sodium acetate and two volumes of absolute ethanol. The DNA was collected by centrifugation method (12).

Polymerase chain reaction amplification of the 16S ribosomal RNA (rRNA) gene of H. pylori- The primers that recognize the *H. pylori*-specific region in the 16S rRNA gene have been described by Ho et al. (20).The polymerase chain reaction amplification analysis and the PCR product from gel was photographed by alpha imager (12, 18).

Statistical analysis

The experimental results were expressed as mean ± SD (standard deviation). The data interpretation was performed with the GraphPad-Instat Software Program (GraphPad-Instat Software Inc., San Diego). Statistical evaluation of data was performed using an analysis of variance (ANOVA) and,

depending on the outcome of the ANOVA (Dunnett's multiple comparison test) the significance of differences was determined. Statistically significant differences between groups were defined as $p< 0.05$.

Results and Discussion

***Morphology and particle size* of microspheres**

Scanning electron micrographs of Cl-loaded and ethyl cellulose coated microspheres morphology are presented in Fig.1 (a) and (b) respectively. F_3 microspheres appeared to be discrete, spherical, free flowing and monolithic matrix type (Fig. 1a). However, after coating with ethyl cellulose batch F_{13} microspheres were white, elastic textures, irregular and comparatively larger in shape (Fig.1b).The diameter of microspheres varied 14±2 and 22±1µm.The SEM photomicrographs indicate that microspheres diameter as observed by optical microscope was significantly higher than that observed under scanning electron microscope. It might be explained by the fact that the incompletely dried microspheres (in swollen state) were observed under optical microscope, whereas the microspheres particles were fully dried when SEM study was performed.

SEM microphotograph reveals that a microsphere of batch F_3 was spherical and the surface was slightly striated and rough which might be attributed to extraction of the drug on the surface of microspheres. In contrast, ethyl cellulose coated microsphere was regular and larger in shape. This may be due to the formation of EC coat on the entire surface, resulting in larger, smooth and shining microspheres.The particle size and size distribution of microspheres was found to be uniform and symmetric in appearance.

Drug entrapment and percent yield

The effect of various parameters on encapsulation efficiency of microspheres has been shown in Table I. Encapsulation was found to be consistently higher for all the batches of the formulation. However, the highest encapsulation efficiency was observed in batch F_3 (83±1.3%). The higher encapsulation efficiency was observed, as concentration of alginate increased which in turn to provide greater availability of active divalent calcium ions (Ca^{+2}) binding sites in the polymeric chains and consequently greater degree of cross linking was resulted in the formation of microspheres. The yields of microspheres were presented to be 65±2 to 76±3 %. The highest yield was observed in batch F_3 of the microparticles. The probable reason behind this may be due to high viscosity of the polymer solution which was retarded the leakage of the drug from dispersion of the polymer and cross linked Ca^{+2} .

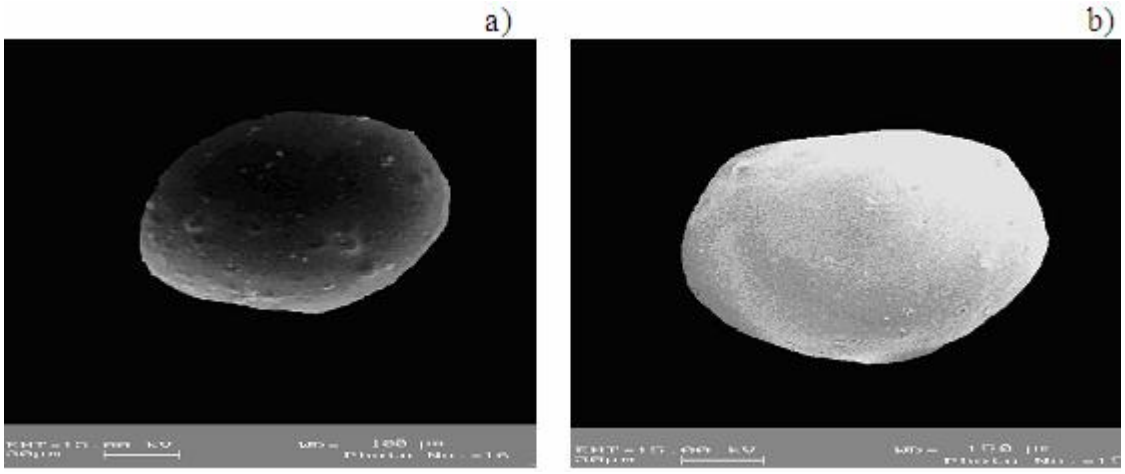

Figure1. Scanning electron microscopy of dried microspheres of a) batch F_3 and b) EC coated batch F_{13}

Buoyancy

Microspheres were steeped in test solution of SGF or phthalate buffer solution pH 3.4 and all the batches were floated in the medium for more than 8 h without any sign of degradation. The buoyancy of microspheres was found to be maximum in F_3 (86 ±3%) and minimum in F_2 (60±4%). However, in acidic fluid, entrapped Ca^{+2} in the microspheres were leakage and leave the pores. Moreover, the pores were filed with air results to impart floating behaviour. The microspheres were more floatable containing higher ratio of sodium alginate with compare to those microspheres contained low concentration of the polymer. This may be attributed to a decrease in density of microspheres with an increase the concentration of the polymer.

Measurement of in vitro drug release

In vitro drug release study of the optimized formulation (batch F_3) and marketed tablet (batch M) was carried out in the SGF solution (pH 1.2) mimicking fasted state, and in phthalate buffer solution (pH 3.4) mimicking fed state for a period of 8 h. Comparative *in vitro* drug release pattern exhibited a biphasic release of the Cl with initial rapid drug release (burst effect) followed by sustained release phase (Fig. 2.a). The drug release from marketed tablet was 89±1% (batch M) and 80±2% (batch M') in SGF and phthalate buffer respectively within 2 h. Cl release from batch F_3 was 47±2 % (SGF) while that of batch F'was 42±2 % (phthalate buffer).The comparative dissolution study of ethyl cellulose coated formulation and the marketed tablet in SGF solution has been shown in Fig. 2(b).

The dissolution profiles exhibited in the fed state (pH 3.4) indicate concomitant administration of meals as an essential aspect to obtain the desired controlled release of the gastroretentive formulation of Cl. The two-way analysis of variance (ANOVA) revealed a significant difference between *in vitro* release profiles of Cl in the fed and fasting state at a 95% confidence interval (p<0.05).Various release kinetic models has been applied to elucidate the mechanism of drug release from the formulation in fasted state. Drug release from the optimized formulation F_3 followed the Higuchi (R = 0.9862, n = 0.39) and Peppas models (R = 0.9842, n = 0.32), respectively, suggesting a diffusion based mechanism of drug release as the diffusion exponent values were less than 0.45[18].

The formulation batch F_{13} exhibited maximum dissolution efficiency of 51 % in 240 min. The dissolution profile of the batch best fitted zero-order release with R value of 0.9832 was optimized as pH stimuli sensitive controlled-release formulation of clarithromycin. On the other hand, F_{33} exhibited the least dissolution efficiency of 46 % after 240 min (Table II). This may be attributed to the fact that lowest ethyl cellulose concentration might have decrease the density of polymer matrix and shortened the diffusion path length that the drug molecules have transverse. When *in vitro* release data of marketed product of Cl was compared with ethyl cellulose coated microspheres containing Cl in SGF (pH 1.2), formulation F_{13} showed better controlled release behaviour. Hence it was selected as optimized formulation for further study.

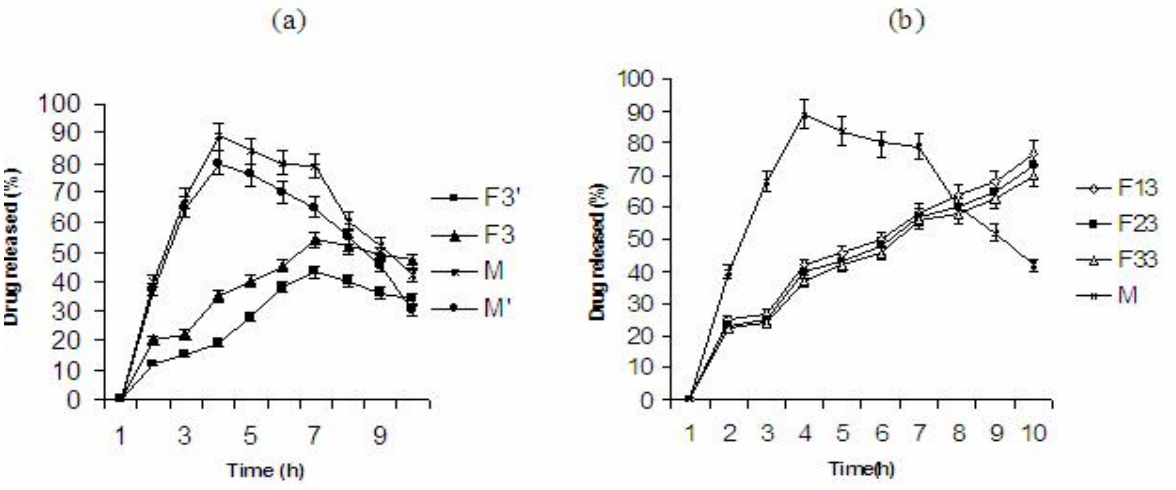

Figure 2. Comparative dissolution study (a) microspheres at different pH and (b) ethyl cellulose coated microspheres and marketed tablet. Points represent mean ± SD (*n* = 3).

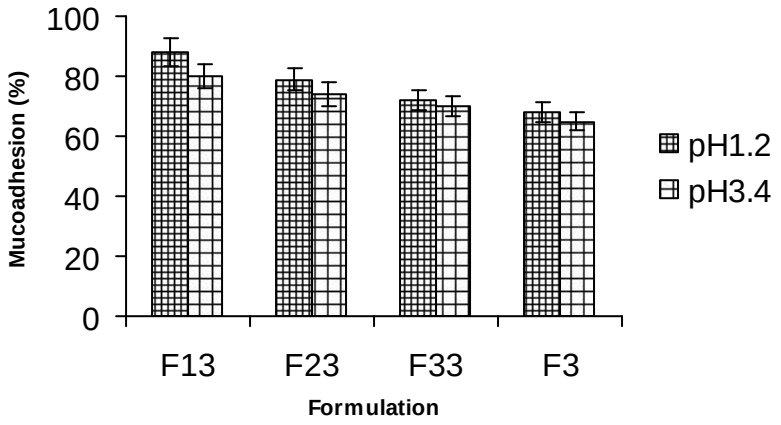

Figure 3. Comparative mucoadhestion study of microspheres. Colums represent mean ± SD (*n* = 3)

Percent mucoadhesion

Mucoadhesive tendency of the selected batch F_3 was compared with ethyl cellulose coated microspheres batches (F_{13}, F_{23} and F_{33}). The sequence of mucoadhesive property was in order of F_{13}> F_{23}> F_{33}> F_3 both in SGF solution (pH 1.2) and phthalate buffer solution (pH 3.4). Maximum mucoadhesion tendency was show by ethyl cellulose coated microspheres (batch F_{13} 88±2 %) in SGF solution while at pH 3.4 (phthalate buffer), mucoadhesion was 80±2% (Fig. 3).

Robinson et al. [19] reported that the solubility, hydration and mucoadhesion nature of the polymers depend on the pH of the gastrointestinal medium. Therefore mucoadhesion study was carried out in SGF (pH 1.2) in empty state and phthalate buffer solution (pH 3.4) fed state. A significant difference (p<0.05) was observed in mucoadhesion percent in both of the solution (pH 1.2 and pH 3.4) for microspheres F_3 and EC coated microparticle batches. This difference in the mucoadhesion may be due to higher affinity of the polymer composite at SGF solution toward glycoproteins of mucosal gel. The mucoadhesive nature of microparticle was found to decrease in phthalate buffer solution at pH 3.4 which would have occurred due to ionization of polar groups like carboxyl groups, hydroxyl groups etc. from the polymers, resulting to increase of solubility and reduction in adhesive strength.

In vitro growth inhibition studies

Susceptibility of clinical isolates of H. pylori - As shown in Table 3, MIC_{50} (the concentration that will inhibit 50 % growth of a *H.pylori* isolate in vitro) and MIC_{90} (the concentration that will inhibit 90 % growth of a *H.pylori* isolate in vitro), of clarithromycin for the isolate *H. pylori* were 24 µg mL^{-1} and 62 µg mL^{-1} respectively. The results indicated that MIC range of the drug was varied between ≤ 0.015 and 200 µg mL^{-1}. Cl is one of the most active and predictable antimicrobial agents against *H. pylori* and has highest eradication rate in monotherapy *in vivo*. Cl has a minimum inhibitory concentration (MIC) of 10 µg mL^{-1} against clinical isolates of *H pylori* and is more active than erythromycin, roxithromycin, and azithromycin. *H. pylori* is fastidious and very slow-growing, it takes 3-4 days to form visible colonies for MIC determination under a microaerophilic atmosphere, and the resulting MIC values are greatly affected by the environmental conditions .

Table 3. Susceptibility of clarithromycin for the clinical isolates of *H. pylori*

Clarithromycin concentration (µg mL^{-1})	**MIC_{50} (µg mL^{-1})**	**MIC_{90} (µg mL^{-1})**	**MIC Range (µg mL^{-1})**	**Susceptibility (%)**
≤ 0.015 - 240	24	62	≤ 0.015 - 200	95.4 %

Percent growth inhibition of H. pylori – Percent growth inhibition by the microspheres was evaluated for both uncoated (F_3) and ethyl cellulose coated (F_{13}, F_{23}, and F_{33} at various time intervals up to 24 h. The antimicrobial efficacy of formulations and free Cl was calculated as the ratio of optical density of mixture of formulation and the inoculums (suspension of *H. pylori*) against that of tubes containing *H. pylori* alone. The *H. pylori* culture tubes containing placebo microspheres (control batch) did not show growth inhibition (4±1% after 5 hr of incubation) in *H. pylori* culture (Fig. 4).

The percentage growth inhibition of batch F_3 was 56±2 % and of EC-coated microparticles F_{13} was 85 ±2% after incubation for 5 h. The culture tubes containing control batch did not show antimicrobial activity, whereas batch F_{13} showed good growth inhibition *in vitro* and the Cl floating microspheres might have effectively targeted the *H. pylori* surface and eradicated the infection.

In vivo *H. pylori eradication*

Effects of repetitive administration of different formulations against gastric infection caused by *H. pylori* in Mongolian gerbils have been shown in Table 4. In the control group receiving no Cl, around 100 viable bacteria colonized in the animal stomach. Control batch showed negligible bacterial clearance while the Cl suspension therapy showed 33% bacterial clearance at the dose of 10 mg kg^{-1}.

The EC- coated batches F_{13} and F_{23} showed 100 % bacterial clearance while batch F_{33} showed 77 % clearance of the bacterial colony. Polymerase chain reaction amplification of extracted gene from the stomach of batch F_{13} treated group of the animals showed absence of amplified bacterial gene (Fig.5b). In contrast to this, PCR microphotograph of the DNA extracted from the gerbil stomach from the control and Cl suspension always showed his presence of the bacterial gene (Fig. 5a).These results clearly indicate that Cl administered in the form of batch F_{13} was more effective in clearing *H. pylori* from gerbils stomach than Cl administered in the form of a suspension.

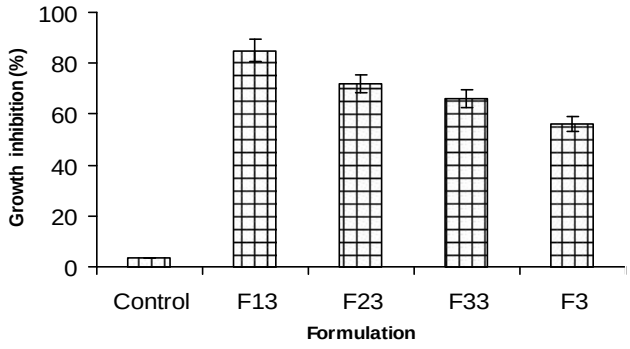

Figure 4. Growth inhibition efficiency of formulated batches incubated for 5 hr with the isolated strain of *H. pylori*. Bars represent mean ± SD (*n* = 3).

Table 4. Different formulations against *H. pylori* in Mongolian gerbils

Formulation Code	Dose (mg Kg-1)[a]	(%)[b] No. of gerbils cleared Infection/total no.100	Bacterial recovery (log CFU per stomach)[c]
Control	0	0/6(0)	8.46 ± 0.3
Cl suspension	10	2/6(33)	4.08± 0.1
Batch F_{13}	10	6/6(100)	ND
Batch F_{23}	10	6/6(100)	ND
Batch F_{33}	10	5/6(77)	3.41± 0.1

CFU - colony-forming unit

ND - colony not detected

[a, b] Clarithromycin (Cl) was administered daily once for 3 days (F_{13}, F_{23} and F_{33} contain equivalent amount of Cl)

[c] Bacterial cell counts less than $10^{1.45}$ CFU were considered to be $10^{1.45}$ to calculate the mean. Means ± SE (n=3)

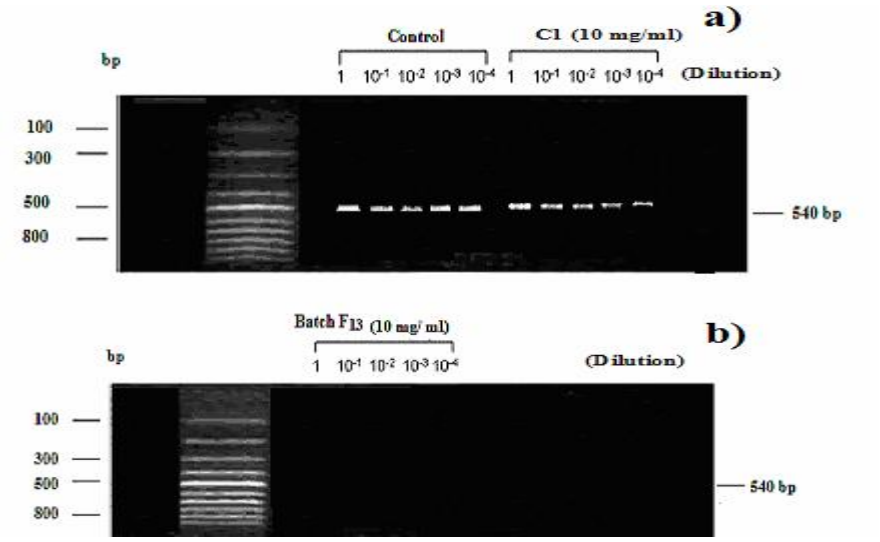

Figure 5. Polymerase chain reaction amplification of the 16 S rRNA genes of *H. pylori* isolated from infected gerbil's stomach: a) pattern of the PCR product after gel electrophoresis in control and clarithromycin suspension treated group and b) batch F_{13} treated group of gerbils stomach. *bp* (base pair) is molecular marker.

These results clearly indicate that short residence time of Cl suspension in the stomach and the concentrations of Cl were not sufficient in gastric mucus layer to eradicate *H. pylori*. Absence of the bacterial DNA in treated gerbils stomach as evidenced from PCR studies indicates the efficacy of F_{13} formulation. (Fig. 5.b). It has also been observed that batch F_{13} was able to release Cl for prolonged period and can maintain MIC of the drug around *H. pylori* surface and for sufficient time period to completely eradicate the bacteria with their genetic material from the infected animal. This data supports the termination of probability for bacterial resistance against antibiotic.

Conclusion

It is concluded from the above study that the developed prototype formulation of clarithromycin combining an excellent mucoadhesive and controlled drug release pattern could possibly be advantageous in term of improved treatment of *H. pylori* infection. The optimized batch F_{13} exbited mucoadhesive and sustained release profile of clarithromycin for a period of at least 8 h. *In vitro* growth inhibition study against *H.pylori* infection has shown good antimicrobial activity in the optimized batch. Implicit to this data it can be concluded that the optimized batch of the formulation containing clarithromycin appears to have potential for targeted delivery of antibiotic in the gastrointestinal tract in order to eradicate *H. pylori* infection. This also suggests that can be a good alternative to microparticulate based system for cure of the infection, which offers pH sensitive drug release, prolongs gastric residence and can prevent degradation of the drug in the gastric pH.

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