Formulation and evaluation of pH sensitive polymeric blended microgel beads of clarithromycine for the effective treatment of *Helicobacter pylori*

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ABSTRACT

The objective of this investigation was to develop a pH sensitive intra gastric floating drug delivery system of clarithromycin in order to treatment of peptic ulcer disease caused by Helicobacter pylori (H. pylori). In the present investigation buoyant beads of sodium alginate, wherein, the oil was entrapped, was blended with gellan gum or pectin in order to evaluate its potential in the targeted sustained delivery of clarithromycine (Cl) in the gastric region. The formulation was developed using inotropic gelation technique using calcium carbonate as gas forming agent and further it was emulsified with mineral oil. The scanning electron microscope photograph indicated that prepared beads were spherical in shape and formulation variables such as ratio of the blended polymer, calcium carbonate effected bead size, floating and encapsulation efficiency of microbeads. The formulation exhibited sustained release profile and was best fitted in the Peppas model of drug kinetic and release exponent (n) value was more than 0.45. Coating of selected formulation exhibited zero-order sustained pattern of the Cl release up to 8 hr. In - vitro growth inhibition study of the coated beads showed good anti microbial activity against isolated H. pylori strain. The Results provides evidence that optimized gel bead may be preferred for gastro retentive controlled delivery of clarithromycine.

Keywords: Clarithromycine (Cl); pH sensitive drug delivery system ; Gastric retention.

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INTRODUCTION

Gastro-retentive systems that can precisely control the release rate of target drug to a specific site of gastro -intestine (GI) have had an enormous impact on the healthcare system. This system are particularly important for design of dosage form for locally active to the gastric mucosa in the stomach in order to termination of mucosal infection of *Helicobacter pylori* [1].

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Helicobacter pylori (H. pylori) are a small, spiral, microaerophilic, gram-negative bacteria and it is recognized to be associated with gastritis and duodenal ulcers. The microorganism has also been reported to be involved in the pathogenesis of other diseases, such as chronic atrophic gastritis, adenocarcinoma of the body or antrum of the stomach, gastro-esophageal reflux disease, peptic esophagitis etc [2]. The bacteria penetrates the gastric mucus layer and fix itself to various phospholipids and glycolipids in the mucus gel. Therefore, access of antimicrobial drugs to the site is restricted from both the lumen of the stomach and the gastric blood supply.

Gastroretentive drug delivery systems like floating and bioadhesive drug delivery systems would improve the therapeutic effects of antimicrobial drugs. The bioadhesive drug delivery system can plug and seal the infected and inflamed mucosal cell lines in the gastrointestine (GI) [3, 4]. Drug formulations that deliver the drug for a prolonged period of time are important constraints for the efficient therapy to achieve spatial placement of the dosage form in the GI. Design of novel gastro retentive, pH sensitive formulation would improve local therapy in the GI.

Gastroretentive systems have gained considerable attention due to their ability to adhere to the mucus layer, as well as to release the drug in a sustained manner in the mucosa of the stomach [5]. Narkar et al. 2010 [6] prepared and evaluated stomach-specific controlled release mucoadhesive drug delivery system prepared by ionotropic gelation of gellan beads, containing acid-soluble drug amoxicillin trihydrate. Rajinikanth and Mishra [7] reported gellan gum based floating beads containing clarithromycin were prepared by iontotropic gelation method for stomach-specific drug delivery against *Helicobacter pylori* and showed good antibacterial action against isolated H. pylori strain. Babu et al. 2010 [8] prepared controlled release gellan gum macro beads of amoxicillin and evaluated its potential for gastro retentive controlled release of the drug.

Clarithromycin (Cl) is a broad spectrum macrolide antibacterial agent that is primarily bacteriostatic and its antibacterial action is due to binding of the 50 S ribosomal subunit of susceptible organism and inhibiting protein synthesis [9, 10].

The proposed new clarithromycine microgel beads were composed of optimized blended ratio of gellan gum or pectin in sodium alginate, was used as carrier polymer .The drug penetrated and freely distributed in the vicinity of the infectious microbe in order to maintain the minimum inhibitory concentration (MIC) for long time in the gastric region. Sodium alginate blended gellan gum or pectin may enhanced the mucosal penetration of Cl that may serve as potential vehicle for the targeting of the antibiotic around the microbial lesion like *H. pylori* . The primary objective of the work was to develop a consistent formulation of Cl that enjoys all the advantages of a floating single unit dosage form but at the same time is devoid of disadvantages of single unit dosage forms, like sticking or being obstructed in the gastrointestinal tract. The release behaviour of the gel beads capable of floating in gastric fluid was investigated with the aim to achieve a gastroretentive, multiple units, and controlled release formulation of clarithromycine.

MATERIALS AND METHODS

Materials

Clarithromycine was obtained as gift sample from Ranbaxy Laboratories Ltd, Gurgoan, India. Gellan gum was purchased from Sigma-Aldrich Chemicals Ltd, New Delhi, India. Pectin, calcium chloride and ethyl cellulose were obtained from S.D. Fine Chem. India. Light mineral

oil was obtained from the Central Drug House, India. All other ingredients, reagents and solvents were of analytical grade.

Preparation of polymeric blend oil entrapped bead

Oil entrapped polymeric blend gel bead of clarithromycine was prepared by ionic gelation method. Aqueous solution of pectin and gellan gum (1.0-1.5 % w/v) was prepared with deionized water and was successively dispersed in to aqueous slurry of sodium alginate (2.0 - 2.5 % w/v) with continues stirrer for 20 minutes. The drug (0.90 % w/v) and calcium carbonate (0.55-1.5 % w/w) were dispersed uniformly into 20 ml of the polymeric blended mixture with continuous stirring until a uniform dispersion was obtained (Table 1).

The mixture was emulsified with 05-15 % w/v of light mineral oil using Silverson emulsifier (Hicon, L5M-4, and India) maintained continuous stirring with the oil at 500 rpm for 5 min. The resultant drug loaded emulsions was dropped through a 21G syringe needle into 100 ml of 0.45 mol ml^{-1} of calcium chloride ($CaCl_2$) solution, which was kept under stirring to improve the mechanical strength of the beads and also to prevent aggregation of the formed beads. Immediate formation of small micro gel beads of clarithromycine loaded sodium alginate blended, either with gellan gum (formulation COB) or pectin (formulation HOB) gel beads took place after 5 minutes of curing time, the formed beads were washed with distilled water and collected and dried at 40°C for 6 hr.

Table 1: Composition of drug loaded polymeric blended gellan gum bead.

Formulation		Drug (% w/v)	Gum (% w/w)	oil (% w/v)	Calcium carbonate (% w/v)
(COB)	(HOB)				
C1	P1	0.90	2.0:1.5	05	0.55
C2	P2	0.90	2.0:1.5	10	0.55
C3	P3	0.90	2.0:1.5	15	0.55
C4	P4	0.90	2.0:1.5	05	1.5
C5	P5	0.90	2.5:1.0	05	0.55
C6	P6	0.90	2.5:1.0	10	0.55
C7	P7	0.90	2.5:1.0	15	0.55
C8	P8	0.90	2.5:1.0	05	1.5

COB = Sodium alginate: gellan gum
HOB = Sodium alginate: pectin
Gum = Sodium alginate: gellan gum/pectin
Oil = Mineral oil

Coating of gel beads

Formulated microgel beds of the batch were selected for optimization in order to further modification of drug release pattern. The coating parameters were 5-10% (w/v) ethyl cellulose (EC) solution in acetone and coating times was fixed (5 - 10 min). Gel beads (2 g) were placed in a fluidized bed dryer (TG 100, Retsch, Germany) and the coating solution was sprayed on the fluidized beads using a spray gun for a period of 10 minute at an air inlet speed of 220 m s^{-1} at room temperature. The beads were dried at room temperature for a period of 24 hr until all solvent was evaporated, leaving a film of EC coat on the gel beads (Table 2).

Table 2: Independent variables of the formulation bead coated with ethyl cellulose

Formulation code	EC concentration % w/v	Time of coating (min)	% drug release $t_{480\ (min.)}$ a	R^2
C_{15}	5	5.0	84± 1.4	0.9844
C_{25}	5	10	74±1.5	0.9835
C_{35}	10	5.0	70±1.6	0.9713
C_{45}	10	10	67±1.4	0.9732

EC = ethyl cellulose
R^2 = correlation coefficient derived from zero order drug release kinetics.
C_{14}, C_{24}, C_{34} and C_{44} = ethylcellulose coated optimized formulation of batch C_{14}.
A = Mean ± SD (n = 3)

Particle size and morphology

Particle size of the prepared beads were determined in three set using an optical microscope (Model BH-2, Olympus, Japan) fitted with a stage and an ocular micrometer. Mean diameter was calculated by measuring diameter of 20 dried beads. The external and internal morphology of micro gel beads were studied by scanning electron microscopy. The micro beads were coated with gold palladium under an argon atmosphere using a gold sputter module in a high vacuum evaporator. The coated samples were then observed with a scanning electron microscope.

In vitro floating study

In vitro floating study was performed using a USP 24 dissolution apparatus II having 500 ml of simulated gastric fluid (SGF), pH 1.2. The medium temperature was kept at 37±0.5 °C. The floating beads (1.0 g beads) were soaked in the dissolution medium and the medium was agitated with a paddle at 50 rpm. After agitation the beads that floated on the surface of the medium and those that settled down at bottom of the flask were recovered separately. The percentage of floating was measured by visual observation [11].

Encapsulation efficiency and drug content

Accurately weighed (100 mg) grounded powder of beads was soaked in 100 ml phosphate buffer (pH 7.5) and allowed to disintegrates completely for 4 hr[11] . The resulting dispersion was sonicated using a probe sonicator (UP 400 s, Dr. Hielscher GmbH, Germany) for 30 min and then filtered through a 0.45 μm filter. The polymeric debris was washed twice with fresh phosphate buffer to extract any adhered drug and drug content was determined spectrophotometrically at 353 nm against constructed a calibration curve. The drug content (DC) was calculated according to Eq 1 . The encapsulation efficiency (EE) was calculated according to the relationship in Eq 1.

(DC) % = (Amount of drug in beads /Amount of beads) × 100 …………….(1)

The encapsulation efficiency (EE) was calculated according to the relationship in Eq 2.

EE (%) = (C/T) x 100 ……………………………………………………… (2)

Where C is the calculated drug content and T is the theoretical drug content.

In -Vitro drug release

In- vitro dissolution studies were performed for all the formulation gel beads using USP 24 dissolution test apparatus II with a basket type [12]. An accurately weighed 50 mg amount of the

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beads were taken in to 900 ml dissolution medium of the simulated gastric fluid (SGF , pH 1.2, fasted state condition) or phthalate buffer solution, pH 3.4 (fed state condition) and maintained at a temperature of 37°C ± 0.5°C and stirred at a speed of 50 rpm. The sample aliquot, a 10 ml was withdrawn at 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 hrs and the volume was replaced with an equivalent amount of the dissolution medium. The collected samples were filtered and suitably diluted and analyzed at 353 nm using a UV-visible spectrophotometer. Additionally, an experimental batch F and FF containing 10 mg Cl and lactose (q.s.) filled in a capsule (# 2) was used as a reference formulation.

Kinetic release evaluations
The drug release data of the studies were analyzed with various release kinetic models like zero order, higuchi and korshmaer-Peppas [13-15], were applied to elucidate the mechanism of drug release from the beads in the fed state. These Kinetic models were used to analysis of the dissolution study date with following equations (3), (4) and (5).
Zero-order model:

$$M_t = M_0 + K_0 t \dots\dots\dots\dots (3)$$

Higuchi model:
$$M_t = M_0 + K_H t^{0:5} \dots\dots\dots\dots (4)$$
Korshmaer-Peppas model:
$$M_t / M\infty = k\,(t)^{\,n} \dots\dots\dots\dots(5)$$

Where M_t is the amount of drug dissolved in time t, M_0 is the initial amount of drug, K_0 is the zero order release constant and K_H is the Higuchi rate constant. $M_t/M\infty$ is the fraction of drug release at time t, k is the release rate constant, and n is the release exponent indicative of the mechanism of release.

In -Vitro growth inhibition studies
The bacterial strain used in this study was originally isolated with gastric biopsy from the peasant suffering with chronic gastritis and peptic ulcer in Institute of Medical Science, Banaras Hindu University Varanasi(BHU) – India. Turbid metric method with slight modification was employed to evaluate growth inhibition [16]. The protocol of the study was approved by Institutional Animals Ethical Committee of the BHU. To suppress the growth of indigenous or exogenous contaminating bacteria, the isolated biopsy sample was grown in brucellla agar (Merck co Germany) containing 10 % horse blood, Vancomycine, polymyxine B and amphotericine and incubated at 37 ^{0} C for 7 days. Isolated sample was subcultured on Brucella agar containing 10 % hoarse blood without antibiotics and incubated at 37 ^{0} C for 3 days in microairofilic condition.

H. pylori strains were grown in brucella broth at 37°C after 7 days in microaerobic atmosphere (5% O2, 10% CO_2, 85% N_2). Growth of the bacteria was monitored by measuring the optical density (OD) of broth cultures with spectrophotometrically at *λmax* 640 nm [17]. The numbers of bacteria were determined in terms of optical density by at a *λmax 640* nm with one optical density unit corresponding to 10^8 colony-forming unit (CFU)/ml. To study the effect of formulations on *H. pylori* growth inhibition, 10 ml of nutrient broth containing *H.pylori* were transferred into sterile test tubes. Plain drug (Cl) and optimized formulation of the ethylcellulose coated batches were taken containing clarithromycine equivalent to 24 µg/ml which is twice in concentration with respect to MIC (12 µg/ml) and added to the tubes and all

the tubes were incubated at 37 °C in a microaerobic atmosphere for 24 hr. The tubes containing culture were shaken at 100 rpm at 37°C in a microaerobic atmosphere condition in incubator for 24 hr. Then 100 μl of nutrient broth of *H.pylori* containing drug and different formulations were removed at various time points (4, 8 and 12 hr) and optical density was determined to assess growth inhibition of the bacteria by counting viable colony using spectrophotometer. The percentage growth inhibition was calculated using the following formula:

$$\% \text{ Growth inhibition} = \frac{OD_{TP} - OD_{TS}}{OD_{TP}} \times 100$$

Where,

OD_{TP} = Optical density of test organism at particular interval.

OD_{TS} = optical density of test mixture at same time interval.

Statistical analysis

The experimental results were expressed as mean ± SD (standard deviation). Statistical evaluation of data was performed using an analysis of variance (ANOVA). The evaluation data was used to assess the significance of differences. Statistically significant difference between the means of batches were defined as P<0.05.

RESULTS AND DISCUSSION

Morphology of microgel bead

The formulation composition and physico-chemical properties of the various batches of Cl floating beads were shown in Tables 1 & 3 respectively. Gel micro beads were produce due to gelation and cross linking of Ca^{+2} ions, provided a gel barrier at the surface of the formulation.

(a)   **(b)**
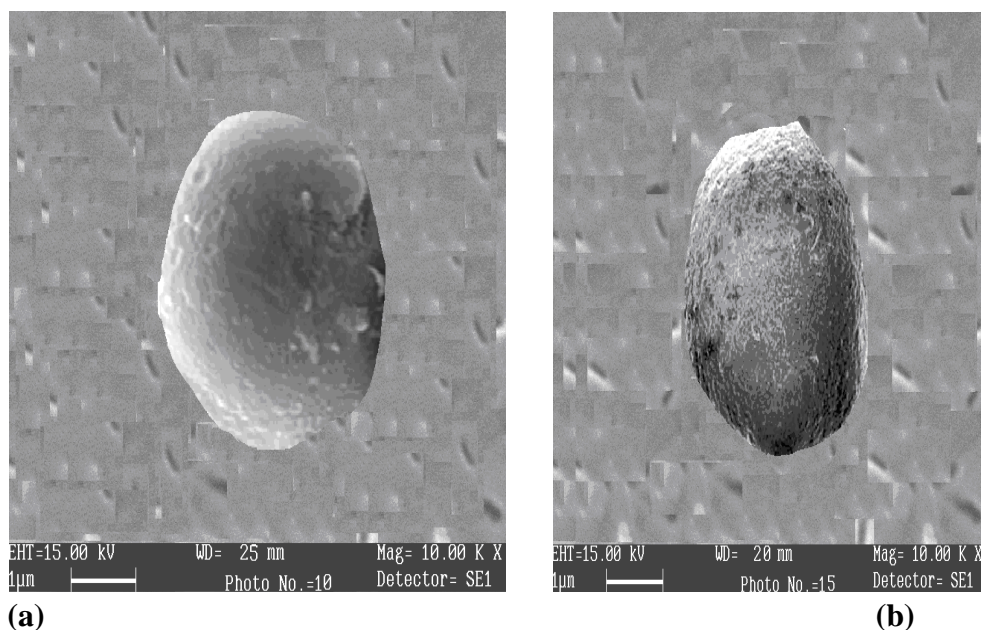
Figure 1: Outer structure of dried oil entrapped blended polymeric bead of (a) batch C_5 and (b) batch P_6 under scanning electron microscope

Scanning electron micrographs (SEM) of Cl loaded oil entrapped sodium alginate bead blended with gellan gum (formulation COB) of batch C_5 or pectin blended (formulation HOB) of batch P_6 are showed in Fig.1 (a) and Fig.1 (b) respectively. Gel bead of the batch C_5 was white, translucent and rigid. The HOB formulation of the batch P_6 was found to be spherical and smooth surface and off -white and elastic. The surface of C_5 floating gel beads appeared smooth and the presence of minor projections on the surface of C_5 gel beads may be attributed to the presence of insoluble drug particles in the bead matrix, which was in contrast to P_6 beads with a smooth surface.

Particle Size of bead
The effect of various formulation parameters on the particle size of prepared floating beads are shown in Table 3. The diameter of COB formulation vary in size 1.50 ± 0.6 mm to 1.93 ± 0.6 mm. while, HOB formulation size was ranges 0.98 ± 0.8 mm to 1.36 ± 0.8 mm. Size distribution pattern of micro beads of both the formulations was showed significance difference as Figure 2 . It has been found that the diameter of beads was significantly increase as the ratio of the sodium alginate increased. This was inferences   that on high concentration of the polymer will increase micro-viscosity of the polymeric dispersion, eventually resulted into formation of bigger beads. Large size beads were form as concentration of calcium carbonate ($CaCO_3$) increased, this is due   to generation of excess Ca^{+2} ions and resulted to formation of weaken and flexible large gel beads.
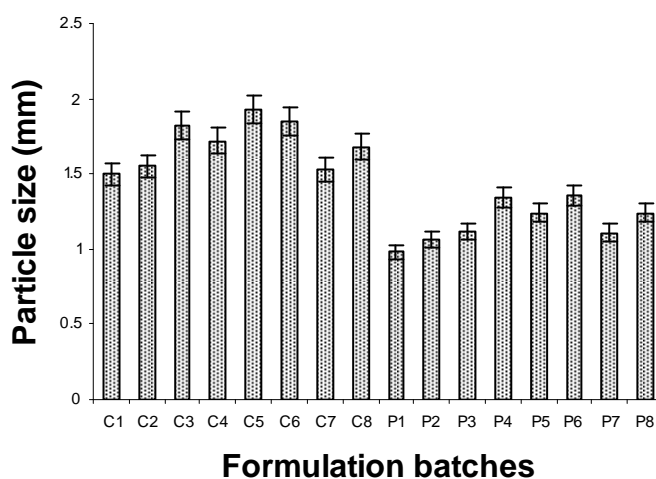

Figure 2: Mean particle size distribution pattern in the different batches of formulations MOB & COB

In vitro floating study
The formulations batch C_5 and batch P_6 were found to be good floating ability (82 ± 1.6 % and 79 ± 1.4 % respectively). The floating ability of the formulation mainly depends on $CaCO_3$ and the polymer used as carrier. On increasing $CaCO_3$ concentration, the percentage floating of micro gel beads were increased due to increase concentration of Ca^{+2} ions consequently the amount of carbon dioxide gas (CO_2) evolved and was responsible for floating of the formulation. The released carbon dioxide is entrapped in the gel network of the formulation, and the gel rises to the surface of the dissolution medium (*in-vitro*) or the stomach [18].

The factors contributing for floating of beads were appeared to be the porous structure of beads, low relative densities of mineral oil (0.84 g ml^{-1}) as compared to that of SGF solution (1.004 g ml^{-1}).

Encapsulation efficiency and drug content

Encapsulation of the drug was found to be consistently higher that is formulation COB (ranges as: 80 ± 0.8 - 97 ± 0.5 %) and formulation of HOB (ranges as: 72 ± 0.2 – $88\pm0.4\%$). Encapsulation efficiency was decreased as the oil concentration was increases due to replacement of the drug from the filled space of the polymer lattice. No significant ($P > 0.05$) effect was observed for $CaCO_3$ concentration on encapsulation efficiency of the prepared gel bead. Batch C_5 of COB formulation was showed highest Cl loading (82 ± 1.8 % w/w) and batch C_3 showed lowest drug loading (62 ± 1.6 % w/w). The HOB formulation of batch P_6 was showed highest drug loading (73 ± 1.4 % w/w) and batch P_2 showed lowest drug loading (51 ± 1.4 % w/w).

Table 3: Characterization of prepared formulation of polymeric blended gellan gum gel beads

Formulatio		Diameter (mm)[a,b]		Floating ability (%)[a]		Encapsulation Efficiency (% w/w)[a,]		Drug content (%)[a, c]	
COB	HOB	COB	HOB	COB	HOB	COB	HOB	COB	HOB
C_1	P_1	1.50 ± 0.6	0.98 ± 0.8	85 ± 1.2	76 ± 1.3	80 ± 0.8	75 ± 0.8	70 ± 1.6	58 ± 1.3
C_2	P_2	1.55 ± 0.6	1.06 ± 0.4	82 ± 1.4	73 ± 1.4	84 ± 0.8	72 ± 0.2	64 ± 1.4	51 ± 1.4
C_3	P_3	1.82 ± 0.8	1.12 ± 0.5	77 ± 1.2	68 ± 1.2	77 ± 0.8	74 ± 0.2	62 ± 1.6	48 ± 1.3
C_4	P_4	1.73 ± 0.4	1.34 ± 0.6	72 ± 1.4	70 ± 1.4	97 ± 0.5	84 ± 0.4	82 ± 1.8	67 ± 1.3
C_5	P_5	1.93 ± 0.6	1.24 ± 0.4	82 ± 1.6	69 ± 1.4	95 ± 0.5	80 ± 0.6	74 ± 1.6	70 ± 1.6
C_6	P_6	1.85 ± 0.5	1.36 ± 0.8	66 ± 1.8	79 ± 1.3	85 ± 0.6	88 ± 0.4	65 ± 1.4	73 ± 1.4
C_7	P_7	1.53 ± 0.8	1.11 ± 0.5	61 ± 1.5	60 ± 1.2	86 ± 0.6	83 ± 0.2	67 ± 1.4	64 ± 1.4
C_8	P_8	1.68 ± 0.6	1.24 ± 0.4	69 ± 1.3	63 ± 1.4	83 ± 0.6	81 ± 0.6	66 ± 1.2	62 ± 1.6

COB   = sodium alginate: gellan gum .

HOB   = sodium alginate: pectin

a   = Mean $\pm SD$ (n = 3)

b   = number of micro beads taken for determination of particle size (n = 20)

c   = Drug content in each 100 mg of bead.
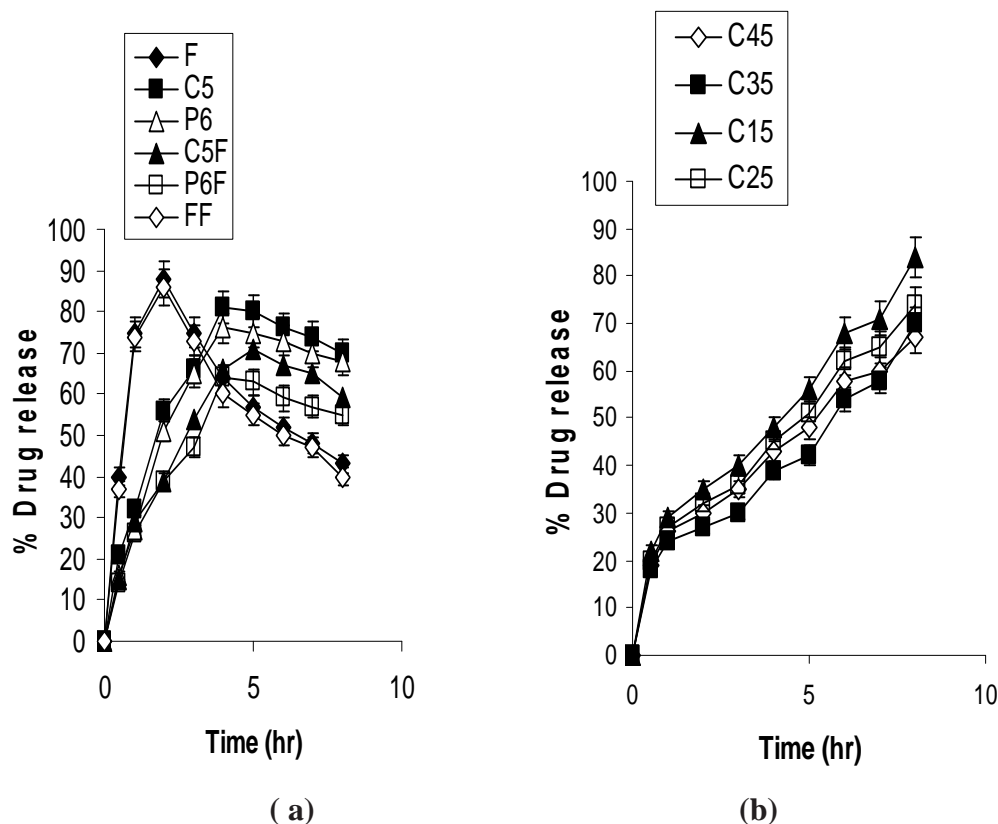
In-Vitro drug release studies

In vitro Cl release study of gel beads was carried out both in the SGF solution and in phthalate buffer for a period of 8 hr. Gel beads were exhibited a biphasic Cl release profile as an initial rapid drug release phase (burst effect) was followed by a slower, gradually declining drug release phase was extended up to 8 hrs (Figure 3a).

Release of Cl from batches C_5 and batch C_5F were 70.0 ± 1.2 % and $59.0 \pm 1.5\%$ in fasted state condition and fed state respectively. While the drug was released within 8 hr from the batches P_6 and P_6F were $68\pm1.3\%$ (SGF, fasted state) and 55 ± 1.4 % (phthalate buffer, pH 3.4). The drug release from experimental reference (capsule was filled with 10 mg of Cl) and the release study was $88\pm1.2\%$ (batch F) in fasted state and $86.9\pm 1.6\%$ (batch FF) in fed state in to within 2 hr and could not sustain the release of the drug over 8 hr , but rather exhibited a rapid first-order decline.

Drug release from the COB formulation of batch C_5 followed the Higuchi ($R^2 = 0.964$) and Peppas models ($R^2 = 0.946$, n = 0.38), suggested a diffusion based mechanism of the drug release as the diffusion exponent values were less than 0.45(19). Behaviour of COB gel beads formulation exhibited a faster release of the Cl while HOB formulation gel beads showed a relatively slow release which may be attributed, higher viscosity of pectin solution than gellan gum and to greater partitioning of the Cl in gellan compared to pectin. Further,

modification of the drug release studies were carried out in formulation COB (Batch C_5) by coating with ethylcellulose (due to stability in gastric pH) (20).


Figure 3: Comparative drug release profile: (a) fasted and fed state condition (b) Ethyl cellulose coated formulation in fed state

The drug release data from the ethyl cellulose coated formulation is given in Table 2. *In vitro* dissolution study of the coated formulation was carried out at fasted state (due to high the drug release rate) with the use of SGF dissolution media. The release data in the coated formulation were showed zero order release pattern (Figure 3b). The release of clarithromycine from batch C_{15} was highest (84± 1.5%) was regarded as an optimized pH sensitive gastroretantive controlled release formulation of clarithromycine.

In -Vitro growth inhibition studies

Percentage growth inhibition against *H. pylori* was evaluated and effect in drug-loaded , drug-free bead (placebo) and plain clarithromycin on the bacterial growth was investigated at various time intervals up to 12 hr. The coated formulation batches ($C_{15,}$ $C_{25,}$ $C_{35,}$ C_{45}) , placebo and plain clarithromycine were selected for *in vitro* growth inhibition studies. Culture tube of *H. pylori* containing placebo bead did not show significant % growth inhibition (2.3±1.2 %) and percentage growth inhibition sequence in the coated formulation was varied C_{15}(80±1.2 %)>C_{25} (77±1.4 %) > C_{35} (75±1.3 %) > C_{45}(72±1.2 %) whereas, plain clarithromycine inhibited hundred percent *H. pylori* growth at 4 hr of the study (Figure 4). The continued incubation of *H. pylori* up to 8 hr the optimized formulation (Batch C_{15}) was eradicated hundred percent growths of the *H. pylori* and further incubation for 12 hr, all the optimized coated batches were shown complete termination of the microbial infection. The results clearly indicate that batch C_{15} is optimized formulation of the study and effectively target on the *H. pylori* surface and eradicate the *H.pylori* from the stomach. Thus,

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it can be expected that the floating formulations with clarithromycin will abolish all the mechanisms of *H. pylori* survival *in vivo* and may provide the better treatment efficacy for *H.pylori* infection
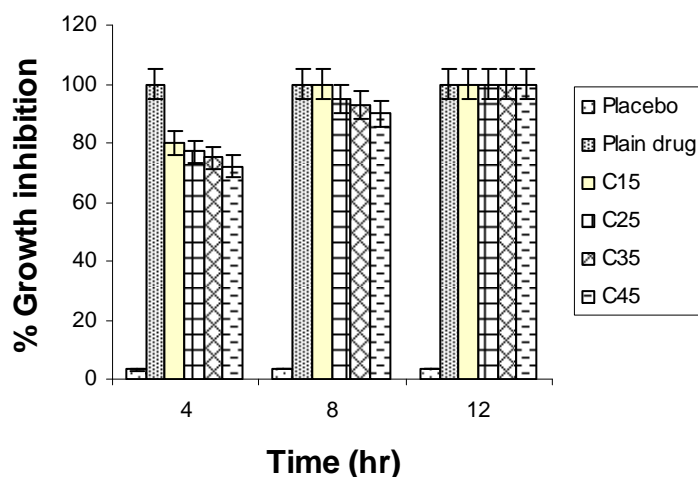
Figure 4. Percentage growth inhibitions of formulations

CONCLUSION

In conclusion, the optimized formulation is anticipated to maintain minimum inhibition concentration (MIC) of clarithromycine at the infection site and potentially allow penetration of the drug inside the mucus gel. This is also anticipated to release the drug so as to eradicate infection lesion in the acidic region of the gastrointestinal tract. Hence the design clarithromycine delivery system that could not only curtail and alleviate the shortcomings of conventional drug delivery vehicles, but also targeted deliver antimicrobial agents like clarithromycine to the infected *H. Pylori* cell lines.

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