Stomach-Site Specific Drug Delivery System of Clarithromycin for Eradication of *Helicobacter pylori*

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Gellan gum based floating beads containing clarithromycin (FBC) were prepared by iontotropic gelation method for stomach-specific drug delivery against *Helicobacter pylori*. The scanning electron microscope photograph indicated that prepared beads were spherical in shape with rough outer surface. Formulation variables such as concentrations of gellan, calcium carbonate and drug loading influenced the *in vitro* drug release characteristics of prepared beads. *In vitro* release rate of clarithromycin was corrected using first order degradation rate constant which is degraded significantly during the release study in simulated gastric fluid pH 2.0. Further, the absence of interactions between drug and polymer was confirmed by differential scanning calorimetry analysis. Kinetic treatment of the *in vitro* drug release data with different kinetic equations revealed matrix diffusion mechanism. Prepared beads showed good anti-microbial activity against isolated *H. pylori* strain. The prepared beads have shown good *in vivo* floating efficiency in rabbit stomach. The stability studies of beads did not show any significant changes after storage of beads at 40 °C/75% relative humidity for 6 months. The preliminary results from this study suggest that floating beads of gellan can be used to incorporate antibiotics like clarithromycin and may be effective when administered locally in the stomach against *H. pylori*.

Key words clarithromycin; floating bead; clarithromycin bead; controlled drug delivery; *Helicobacter pylori*

Oral administration is always the preferred means of drug delivery for systemic circulation and local action in stomach. Many attempts have been made to develop sustained release preparations with extended clinical effects and reduced dosing frequency. A problem frequently encountered with conventional sustained release dosage forms is the inability to increase their residence time in the stomach and proximal portion of the small intestine.[1,2] Gastroretentive drug delivery system is an approach to prolong gastric residence time, thereby resulting in improved oral bioavailability of the basic drugs that have poor solubility in higher pH. Floating drug delivery system (FDDS) and bioadhesive drug delivery are the widely used techniques for gastroretention.[3—5]

Floating oral dosage forms are expected to remain buoyant upon the gastric contents and to consequently enhance the bioavailability (drugs with a narrow absorption window) of all drugs which are well-absorbed from the upper gastrointestinal tract. These systems are also appropriate for drugs which are locally active to the gastric mucosa in the stomach, in particular case of antibiotic administration for *Helicobacter pylori* eradication in the treatment of peptic ulcer disease.[6]

H. pylori is a small, spiral, gram negative organism which colonizes on gastric mucosa of human stomach and produces a serious gastro duodenal disease—including peptic ulcers, gastric lymphoma and acute chronic gastritis.[7] Although *H. pylori* is highly sensitive to most antibiotics, its eradication from patients requires high concentration of drugs to be maintained within the gastric mucous for a longer duration. Thus it can be expected that local delivery of narrow spectrum antibiotics through a site-specific or gastro-retentive drug delivery system may result in complete removal of the organisms in the fundal area of the gastric mucosa due to bactericidal drug levels being attained in the area, and might lead to better treatment of peptic ulcer disease.[8] One way to bring out the complete eradication of *H. pylori* is to treat with one or more antibiotics combined with an anti-secretory agent but these regimens are not fully effective because of patient compliance, side effects and bacterial resistance problems.[9] Other than the multi-antibiotic therapy, different therapeutic strategies have been examined to completely eradicate *H. pylori* from the stomach.[10,11]

Another way to enhance the eradication rate of *H. pylori* is to extend the residence time of the antibiotics in the stomach. The longer residence time of dosage forms will allow more of the antibiotic to penetrate through the gastric mucus layer to act on *H. pylori* where *H. pylori* exists thereby improve the therapeutic efficacy. Moreover, the absorption of an antibiotic into the mucus through the mucus layer (from the gastric lumen) is believed to be more effective for *H. pylori* eradication than absorption through the basolateral membrane (from blood).[9,12]

In order to enhance the efficacy of anti-*H. pylori* agents, we have made an attempt to develop gellan based floating beads for stomach site specific controlled delivery of clarithromycin for treatment of *H. pylori* infection using calcium carbonate as gas forming agent. In this study, calcium carbonate was used as gas-forming agent, dispersed in a gellan matrix. The calcium carbonate present in the formulation, releases carbon dioxide in the gastric environment, thereby making the formulation buoyant, thus prolonging the residence time. The floating gellan beads are multiple-unit systems which may be more advantageous than single-unit systems by avoiding "all or-none" emptying from the stomach during migrating myoelectric complex (MMC) motility of the stomach.[13]

Clarithromycin is a macrolide, orally absorbed, broad-spectrum antibiotic. It is widely used in a standard eradication treatment of gastric *H. pylori* infection combined with a second antibiotic and an acid-suppressing agent.[10,14] The antibiotic with the highest eradication rate in monotherapy *in vivo* is clarithromycin. Clarithromycin has highest eradica-

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tion rate in monotherapy *in vivo*.[10] Gellan gum is a bacterial anionic deacetylated polysaccharide secreted by *Pseudomonas elodea*. Due to its characteristic property of temperature-dependent and cation-induced gelation, gellan was selected as model polymer for preparation floating beads in the formulation.[15]

Experimental

Materials Clarithromycin was generous gift sample from Ranbaxy Laboratories Ltd. (Gurgoan, India). Gellan gum (gelrite) was purchased from Sigma-Aldrich Chemicals Ltd. (New Delhi, India). Brain heart infusion, fetal calf serum, and *Campylobacter* selective media (Skirrow Supplement) were purchased from Himedia, India. All other reagents were of analytical grade.

Methods. Preparations of Floating Beads of Clarithromycin The beads were prepared by ionotropic gelation technique. Gellan solution (0.25—1.0% w/v) was prepared by dissolving the gellan in deionised water and heating at 90 °C. An appropriate amount of drug and calcium carbonate were dissolved/ dispersed uniformly into 50 ml of gellan solution below 40 °C with continuous stirring. The stirring was continued after complete addition until a uniform dispersion was obtained. The resultant homogeneous bubble free slurry dispersion was dropped through a 21G hypodermic syringe needle into 100 ml of ionotropic medium (1.5% w/v of calcium chloride solution), which was kept under stirring condition to improve the mechanical strength of the beads and also to prevent aggregation of the formed beads. Immediate formation of small gelled beads took place, after 10 min of curing time. The formed beads were collected by filtration and dried at 40 °C.

Morphology and Particle Size Analysis Particle size of the prepared beads were determined using an optical microscope (Model BH-2, Olympus, Japan) fitted with a stage and an ocular micrometer. Twenty dried beads were measured for calculating mean diameter. The shape and surface morphological examination of the dried beads were carried out by scanning electron microscopy (SEM-JEOL Model 8404, Japan at magnification 500×).

***In Vitro* Floating Properties of Gellan Beads** The *in vitro* floating study was determined by reported method[16] using USP dissolution apparatus II having 500 ml of simulated gastric fluid (SGF, pH 2.0). The medium temperature was kept at 37±0.5 °C. The floating gellan beads (1.0 g beads) were soaked in the dissolution medium and medium was agitated by 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.

Measurement of Encapsulation Efficiency of Beads The content of clarithromycin in gellan beads was determined by as discussed earlier.[17] Briefly, accurately weighed (100 mg) grounded powder of beads was soaked in 100 ml phosphate buffer (pH 7.4) and allowed to disintegrate completely for 4 h. The resulting solution/dispersion was sonicated using probe sonicator (UP 400 s, Dr. Hielscher GmbH, Germany) for 30 min and then filtered through 0.45 μm filter. The polymeric debris was washed twice with fresh phosphate buffer to extract any adhered drug and the drug content was determined by reversed-phase high performance liquid chromatography (RP-HPLC) method. The apparatus used for HPLC analysis was an Agilent 1100 quaternary pump, with a variable wavelength detector, thermostatted autosampler and column thermostat. A Hypersil ODS C18 column (250 mm×4.6 mm i.d., 5 μm, Thermo, U.K.) was fitted with a Phenomenex guard column packed with octadecyl C18 (Phenomenex, U.S.A.). The mobile phase consisting of acetonitrile–aqueous 0.05 M phosphate buffer solution of pH 4.0 (40 : 60 v/v). The column temperature was maintained at 40 °C and flow rate of 1ml/min. The detection was done on UV detector at 210 nm.[18] Each study was conducted in triplicate. The entrapment efficiency (EE) was calculated according to the relationship:

$$\%\ \mathrm{EE} = \frac{\text{amount of added drug} - \text{amount of non encapsulated drug}}{\text{amount of added drug}} \times 100$$

Measurement of *in Vitro* Drug Release The release of clarithromycin from the floating beads was determined using USP dissolution test apparatus I (USP 24) with a paddle stirrer at 50 rpm. The dissolution medium was 500 ml of simulated gastric fluid (pH 2.0) and the temperature was maintained at 37±0.5 °C. Samples were taken at appropriate time intervals and replaced the same volume of freshly prepared dissolution medium. The samples were assayed by RP-HPLC method as described previously. These experiments were conducted in triplicate.

Degradation of Clarithromycin in pH 2.0 The degradation rate of the clarithromycin in SGF pH 2.0 was determined as described by ref. 10 with slight modification. A known amount of clarithromycin was added to the medium, which was preheated at 37 °C, to make a final concentration of 10 μg/ml. An aliquot of the medium was withdrawn at predetermined time intervals and neutralized with a NaOH (0.05 M) solution in order to prevent the further degradation before being quantified by HPLC.

Differential Scanning Calorimetry (DSC) Analysis Differential scanning calorimetry (DSC) was performed on pure drug, placebo beads and drug-loaded beads. DSC measurements were done on modulated DSC (Q 1000 TA equipped with software Pyris 6.0). About 3.0 mg of sample were placed in aluminium pans and then hermetically sealed with aluminum lids. The thermograms of samples were obtained at a scanning rate of 5 °C/min over a temperature range of 40 to 250 °C under inert atmosphere flushed with nitrogen at the rate of 20 ml/min. All tests were performed twice.

***In Vitro* Growth Inhibition Studies** The bacterial strain used in this study was originally isolated from a human patient (age 50 years) with gastric ulcer in SS hospital, Banaras Hindu University, Varanasi, India. *In vitro* growth inhibition studies were performed using a broth culture of *H. pylori*. *H. pylori* broth culture was made in brain-heart infusion containing 0.25% yeast extract and 10% fetal calf serum and supplemented with 0.4% *Campylobacter* selective supplement (Skirrow supplement). *H. pylori* strains were grown in brucella broth at 37 °C after 7 d in microaerobic atmosphere (5% O_2, 10% CO_2, 85% N_2). Growth of the bacteria was monitored by measuring the optical density (OD) of broth cultures spectrophotometrically (Shimadzu UV-Visible 1601) at 600 nm.[19,20] The numbers of bacteria were determined in terms of OD by UV/Visible spectrophotometer at 600 nm with one optimal 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. Clarithromycin plain drug and different formulations were added to the tubes, and all the tubes were incubated at 37 °C in a microaerobic atmosphere for 24 h.

The tubes containing culture were shaken at 100 rpm at 37 °C in a microaerobic atmosphere condition in incubator for 24 h. Then 100 μl of nutrient broth of *H. pylori* containing drug and different formulations were removed at various time points (4, 8, 12, 24 h) and serial dilutions were plated on modified Skirrow's medium. The agar plates were incubated for 4 d at 37 °C under microaerobic conditions in GasPak (BD Diagnostic Systems, Sparks, MD, U.S.A.). The viable cell counts for each sample were calculated by counting the number of colonies on the agar plates. The colonies were identified as *H. pylori* by morphology and urease activity.[4] The number of colonies per plate was counted and expressed as log CFU in term of OD value. Quantitation of *in vitro* antibacterial activity of formulations was approached in terms of percentage growth inhibition (GI). The percentage growth inhibition (%GI) was calculated using the following formula:

$$\%\ \mathrm{GI} = \frac{\left(\begin{array}{c}\text{OD of test organism at a particular time interval} \\ - \text{OD of test mixture at same time interval}\end{array}\right)}{\text{OD of test organism at a particular time interval}} \times 100$$

***In Vivo* Floating Efficiency (X-Ray) Study** The *in vivo* study was carried out by administering floating beads to rabbits and monitoring them by a radiological method. Six healthy albino rabbits of either sex, weighing 2—2.4 kg (2.2±0.3 kg) were used for the present study. The animals were housed in individual cages, and the experiments were conducted in a sanitized room at a temperature maintained at around 24 °C. Food was withdrawn 12 h prior to the study with water *ad libitum*. To make the beads radiopaque, 1.5 g of barium sulfate was incorporated into polymeric solution (the same formulation composition of FBC2 was used to prepare radiopaque beads) and radiopaque beads were prepared using a similar procedure to that mentioned in the preparation of beads. Twenty beads were administered through oral gastric tube with 25 ml water in fasted state. Afterwards, the animals were not allowed to eat or drink throughout the study (up to 6 h). The beads loaded with barium sulfate showed the same *in vitro* buoyancy as the unloaded units (data not shown). At every hour interval, 10 ml of water was administered to animals throughout the study. Before taking X-ray photographs, the animals were placed in upright posture. The position of the beads in the rabbit's stomach was monitored by X-ray photographs (Siregraph-B, Siemens, Germany) of the gastric region at different time intervals (at 1, 4, 6 h) for 6 h. The number of beads that remained buoyant on the surface of the gastric content and that of all the beads remaining inside the

stomach (buoyant and non-buoyant) were observed visually from the X-ray photographs. The protocol of the study was approved by Animals Ethical Committee of the Banaras Hindu University (Varanasi, India).

Stability Studies To assess long-term stability[21] of prepared floating beads of clarithromycin (FBC2) were placed in hard gelatin capsules and sealed in aluminum packaging coated inside with polyethylene. The studies were performed at 40 °C/75% relative humidity (RH) in the stability chamber (Stability Chamber, Narang Scientific Instruments, New Delhi, India) for 6 months. At the end of the storage period, the formulations were observed for physical appearance, size, shape, surface morphology, drug content and *in vitro* drug release characteristics.

Statistical Analysis 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 evaluation data were used to assess the significance of differences. Statistically significant difference between the means of batches were defined as $p<0.05$.

Result and Discussion

Beads Size and Morphology of Beads The formulation composition and physico-chemical properties of the various batches of the prepared clarithromycin floating beads are shown in Tables 1 and 2 respectively. Scanning electron micrographs (SEM) of clarithromycin-loaded gellan beads and their surface morphology are shown in Figs. 1a and b, respectively. SEM microphotograph shows that beads are spherical in shape with rough outer surface. The mean diameter of gellan beads are shown in Table 2. It has been observed that the diameter of beads increased significantly ($p<0.05$) by increasing polymer, drug and calcium carbonate concentration. This could be attributed to increase in microviscosity of the polymeric dispersion due to increasing concentration of gellan, which eventually led to formation of bigger beads. Furthermore, it was also observed that there was no significant differences in beads size with different concentration of calcium chloride used as cross linking agent for formulation of beads.

***In Vitro* Floating Properties** The floating ability of the prepared formulations was evaluated in SGF (pH 2.0). The time the formulation took to emerge on the medium surface (floating lag time) and the percentage floated on the dissolution medium surface were evaluated and are shown in Table 2. Upon contact with an acidic medium, gelation and cross linking by Ca^{++} ions occurred to provide a gel barrier at the surface of the formulation. The calcium carbonate effervesced, releasing carbon dioxide and calcium ions. The released carbon dioxide is entrapped in the gel network, which is invariably a three dimensional network, producing a buoyant formulation. The floating ability of the formulation mainly depends on calcium carbonate and gellan concentrations. The control beads (without calcium carbonate, FBC5) sank uniformly in the medium. The beads containing 0.5 to 2.0% of the gas-forming agent (calcium carbonate) demonstrated good floating ability (72—99% of floating). The buoyancy lag time for this system was in the range of 5—10 min. The lowest level of calcium carbonate which produced buoyant beads for the duration of drug release study was found to be 0.5% (w/v) at all polymer levels.

On increasing the calcium carbonate concentration, the floating lag time was reduced and duration of floating was increased. The increase in the amount of Ca^{++} and consequently the amount of CO_2 evolved are responsible for the observed reduction in floating lag time and increased duration of floating. Similarly an increase in the polymer concen-

Table 1. Formulation Variables of the Prepared Floating Beads of Clarithromycin

Batch code	Gellan concentration (%, w/v)	Calcium carbonate concentration (%, w/v)	Calcium chloride concentration (%, w/v)	Clarithromycin concentration (%, w/v)
FBC1	0.25	1.00	1.50	0.50
FBC2	0.50	1.00	1.50	0.50
FBC3	0.75	1.00	1.50	0.50
FBC4	1.00	1.00	1.50	0.50
FBC5	0.50	0	1.50	0.50
FBC6	0.50	0.50	1.50	0.50
FBC7	0.50	2.00	1.50	0.50
FBC8	0.50	1.00	0.50	0.50
FBC9	0.50	1.00	5.00	0.50
FBC10	0.50	1.00	1.50	0.1
FBC11	0.50	1.00	1.50	2.00

Table 2. Physico-Chemical Characteristics of Prepared Floating Beads of Clarithromycin

Batch code	Diameter of beads (mm) (mean±S.D.)[a]	Encapsulation efficiency (%, w/w) (mean±S.D.)[b]	Floating ability (%) (mean±S.D.)[b]
FBC1	0.86±0.021	76.30±2.49	88.25±2.32
FBC2	0.89±0.032	81.81±2.98	86.49±2.17
FBC3	0.94±0.041	87.21±2.52	87.44±1.78
FBC4	0.98±0.022	89.25±2.65	86.47±2.19
FBC5	0.81±0.016	75.78±3.52	No
FBC6	0.93±0.023	73.71±2.80	72.56±1.92
FBC7	1.18±0.024	78.80±4.12	99.42±2.54
FBC8	0.88±0.018	77.72±3.58	91.36±2.12
FBC9	0.81±0.014	71.83±1.69	92.38±2.19
FBC10	0.86±0.013	78.79±4.84	88.23±2.78
FBC11	0.92±0.023	88.65±3.87	87.52±2.85

a) $n=20$, *b*) $n=3$.

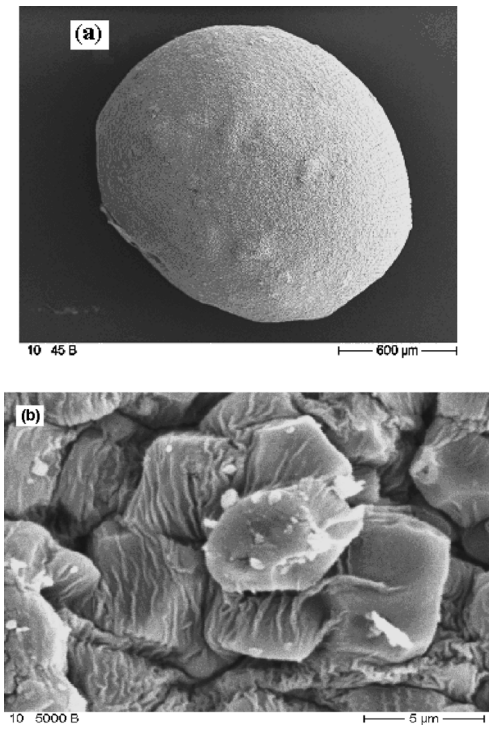

Fig. 1. SEM Photograph of Prepared Floating Beads of Clarithromycin: SEM of Prepared Beads (a), Surface Morphology of Prepared Beads (b)

tration resulted in decreased lag time and an increase in floating time of the prepared beads. Various drug loadings did not produce any significant effect on floating properties.

Drug–Polymer Interaction Studies In an effort to investigate the possible physical and chemical interactions between drug and polymer, we have analyzed: (a) pure clarithromycin, (b) placebo beads, (c) clarithromycin-loaded beads using modulated DSC and the results are displayed in Fig. 2. In DSC thermogram shown a sharp endothermic peak at 227.72 °C for pure clarithromycin due to the melting point of the drug (Fig. 2a). The thermogram of placebo beads and drug loaded beads were shown in Figs. 2b and c, respectively. In placebo beads, thermal transition at 247.07 °C can be seen which is attributed to the glass transition temperature (T_g) of the polymer. The DSC thermogram of the drug loaded beads, the T_g of the polymer was observed at 247.07 °C and endothermic peaks at 226.8 °C was observed as the melting point for the drug (Fig. 2c). During the formulation process, dissolving and heating of the polymer probably caused the T_g to 247.07 °C, but process does not affect the nature of the polymer. The evaluation of the thermograms obtained from DSC revealed no physical interaction between the polymer and the drug in the prepared beads. The results from DSC therograms are clearly indicating that there is no interaction between polymer and drug in prepared floating beads of clarithromycin.

Encapsulation Efficiency The effect of various formulation parameters on the encapsulation efficiency of prepared floating beads are shown in Table 2. Encapsulation was found to be consistently higher for all the batches of beads prepared were found to be in the range of 73.71±2.80—88.65±3.87. Similar high encapsulation efficiencies were achieved for various model drugs having low solubility.[22—24] This may be due to the low solubility of clarithromycin in the calcium chloride solution, which prevents drug partitioning during bead formation. However, the entrapment efficiency of beads increased progressively with increasing polymer concentration as shown in Table 2. This is because of in-

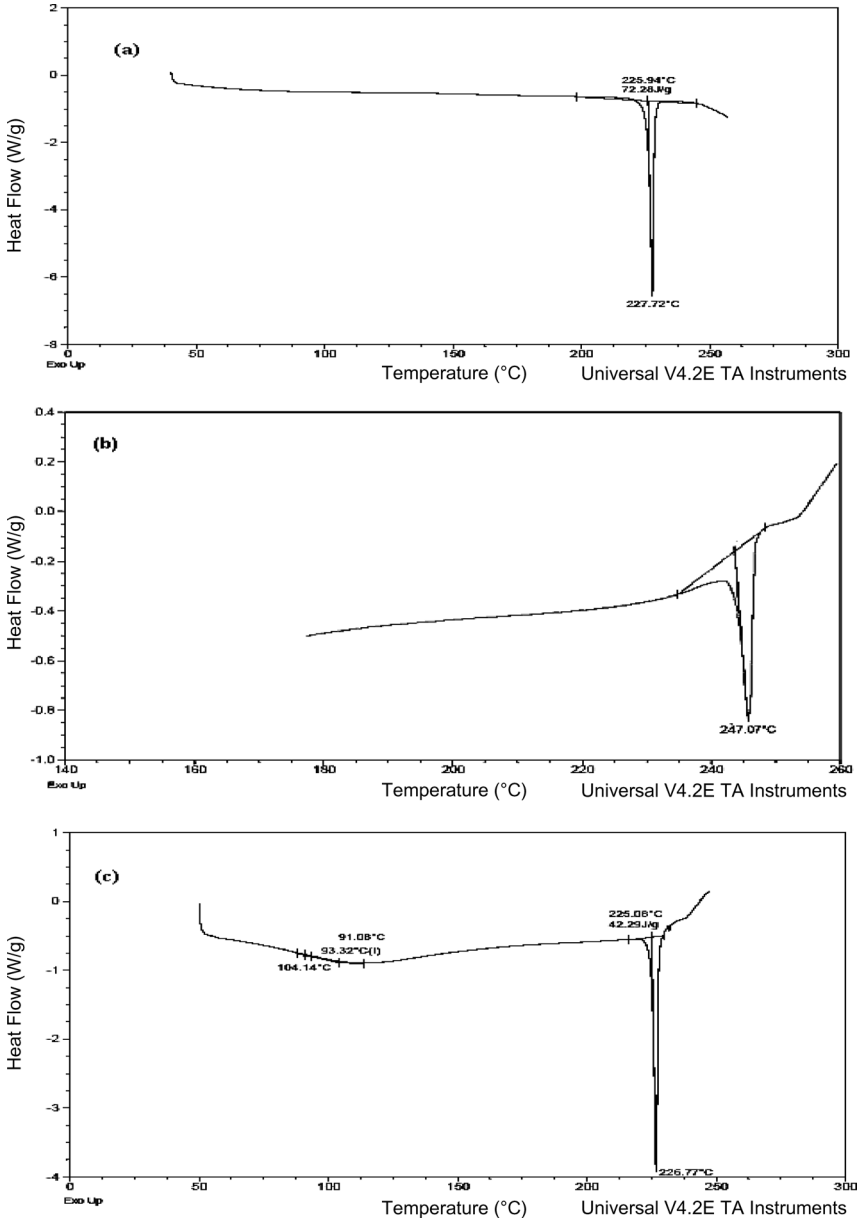

Fig. 2. Differential Scanning Calorimetric Thermograms of Clarithromycin Pure (a), Placebo Beads (b), Drug Loaded Beads (c)

crease in the gellan concentration resulted in the formation of larger size of beads entrapping more amount of the drug. Further, increasing the drug loading from 0.5 to 2% w/v increased encapsulation efficiency marginally. No significant ($p>0.05$) effect was observed with calcium carbonate and calcium chloride on encapsulation efficiency of beads.

Degradation Rate of Clarithromycin in pH 2.0 The degradation and half life of clarithromycin was determined in SGF pH 2.0. Clarithromycin was reported to be unstable in mediums with low pH.[25] Therefore, the results obtained from the dissolution study will underestimate the amount of the drug released from the *in situ* gels. Hence, in order to calculate correct amount of the drug released the degradation rate constant need to be determined. The degradation of clarithromycin was assumed to follow pseudo-first order kinetics, which is described by the following equation:

$$C=C_0e^{-kt}$$

in which C is the concentration of clarithromycin remaining at time t, C_0 is the initial concentration of clarithromycin, and k is the pseudo-first order degradation rate constant. The half-life ($t_{1/2}$) of clarithromycin was determined from the pseudo-first order degradation rate constant. The degradation rate constant and the degradation half-life of the clarithromycin in SGF pH 2.0 were found to be 0.4812 h^{-1} and 1.44 h respectively.

***In Vitro* Drug Release** The *in vitro* drug release profiles of gellan floating beads with different polymer concentrations are shown in Fig. 3. As the gellan concentration of the prepared floating beads increased, the rate and extent of drug release was decreased significantly ($p<0.01$). This could be attributed to increase of gellan matrix density and increase in diffusion path length which the drug molecules have to traverse (by formation of bigger size of beads). The release of drug from these beads was characterized by an initial phase of high release (burst effect) due to good solubility of CAM low pH. However, as gelation proceeds (cross linking of gellan with Ca^{2+} ions from calcium carbonate), the remaining drug was released at a slower rate followed by a second phase of moderate release. This bi-phasic pattern of release is a characteristic feature of matrix diffusion kinetics.[26]

Figure 4 indicates the effect of calcium carbonate concentrations on the release characteristics of clarithromycin from gellan beads. With the increase in calcium carbonate content the release of clarithromycin from the gellan matrix was prolonged. This effect may be due to internal ionotropic gelation effect of calcium carbonate.[27,28] In acidic medium, the calcium carbonate dissolves and the ionized Ca^{++} ions then promote internal gelation by cross-linking with the gellan and retard the drug release from gellan matrix. In control beads (beads without $CaCO_3$), gelation was due to the presence of H^+ ions in dissolution medium whereas the beads containing $CaCO_3$ the gelatin occurs due to Ca^{++}. Although the gelation occurs by monovalent ions (H^+) but it was much less effective than divalent cation (Ca^{++}) in retarding drug release.[29]

The effect of calcium chloride concentration on drug release is shown in Fig. 5. The cross-linking/ionotropic gelation of gellan matrix with calcium chloride is well established and documented.[30,31] The principle of cross-linking or gelation of sodium alginate with calcium chloride is based on the formation of double helical junction zones followed by aggregation of double helical segments to form a three-dimensional network by complexation with cations.[30] In the present study calcium chloride at 3 different concentrations (0.5, 1.5, 5.0% w/v) were used. The results indicate that as

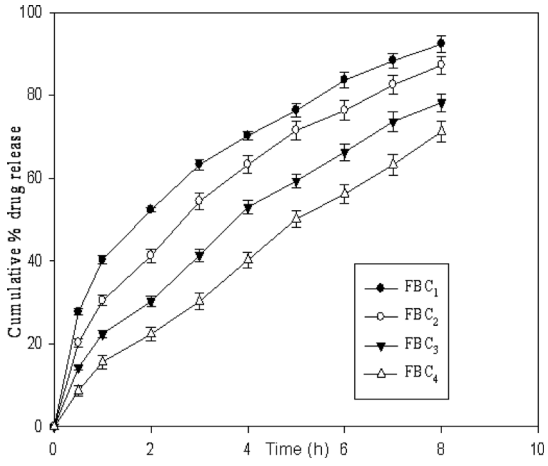

Fig. 3. Effect of Polymer Concentrations on *in Vitro* Clarithromycin Release from Gellan Floating Beads

Bars represent mean±S.D. ($n=3$).

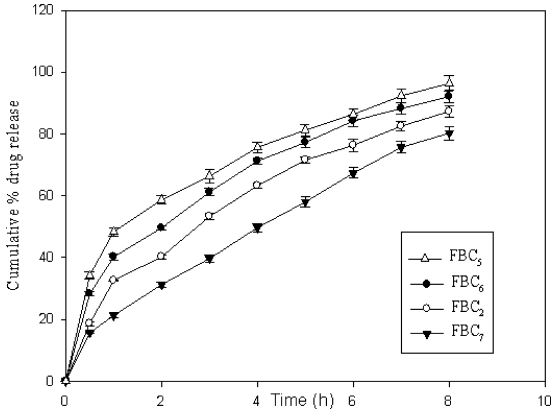

Fig. 4. Effect of Calcium Carbonate Concentrations on *in Vitro* Clarithromycin Release from Gellan Floating Beads

Bars represent mean±S.D. ($n=3$).

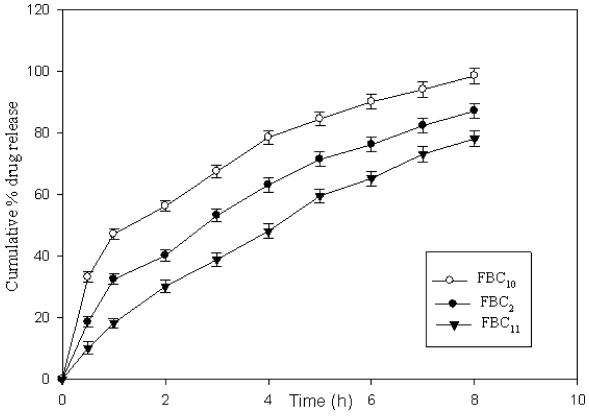

Fig. 5. Effect of Cross-Linking Agent Concentrations on *in Vitro* Clarithromycin Release from Gellan Floating Beads

Bars represent mean±S.D. ($n=3$).

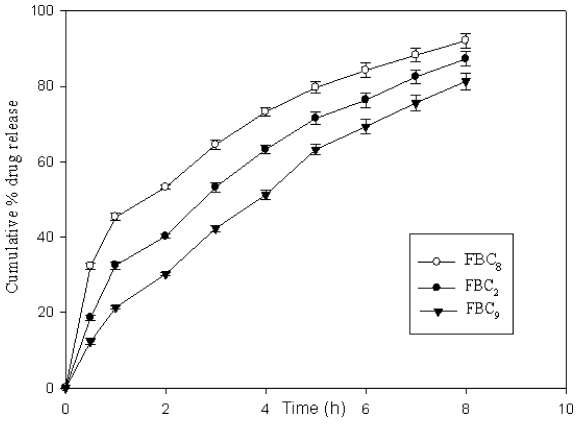

Fig. 6. Effect of Drug Loading on *in Vitro* Clarithromycin Release from Gellan Floating Beads

Bars represent mean±S.D. (n=3).

the concentration of the cross-linking agent increased there was a significant decrease ($p<0.05$) in the drug release. The three-dimensional network by complexation with cations, decrease the porosity of gellan matrix thereby slowed down the drug diffusion from matrix.

The effect of drug loading on *in vitro* clarithromycin release from floating beads was shown in Fig. 6. The results shown in Fig. 6 indicated that rate and extent of drug release was significantly ($p<0.05$) increased from the beads with increasing in the concentration of clarithromycin. The initial burst effect also observed higher for batch (FBC11) containing higher concentration of drug. This is probably due to higher solubility of drug in low pH of dissolution medium.[32]

The release data from floating beads over the whole time period were analyzed according to the treatment proposed by Higuchi (Higuchi, 1962)[33] for drug release from beads containing dissolved drug. In order to investigate the mechanism of drug release the data were fitted to models representing zero-order and first order. The examination of the coefficient of determination (r^2) indicated that drug release followed diffusion controlled mechanism from the prepared beads. The r^2 value for Higuchi's square root of time was always higher in comparison to zero as well as first order (Table 3). Since the release from the prepared beads followed a biphasic profile, it was decided to use a more stringent test in order to distinguish between the mechanisms of drug release. The release data were fitted to Peppas exponential model[34] $M_t/M_\infty=Kt^n$, where M_t/M_∞ is fraction of drug released after time 't' and 'K' is kinetic constant and 'n' is release exponent which characterizes the drug transport mechanism. The values 'n' were in the range of 0.5489—0.7356 (Table 3) indicating that all the prepared formulations followed non-Fickian diffusion controlled mechanism of drug release.

***In Vitro* Growth Inhibition Studies** In order to determine the antimicrobial activity of beads against *H. pylori*, the effect of different drug-loaded, placebo (drug-free) beads and plain drug on *H. pylori* growth was investigated at various time intervals for up to 24 h and results are shown in Fig. 7. The antimicrobial activity of formulations and plain drug are shown in Fig. 7. The antimicrobial effect of formulations and plain drug was determined in terms of percentage growth inhibition (%GI) that was calculated as the ratio of optimal density (OD) of a given mixture against that of tubes containing *H. pylori* alone. The *H. pylori* culture tubes containing placebo beads (control batch) does not show any growth inhibition (maximum % GI was found to be 3.98±1.28 at the end of 4 h of incubation) in *H. pylori* culture (Fig. 7), which suggested that ingredients which are used in formulation have no antimicrobial activity. The percentage of GI value formulation batches FBC2 and FBC4 were found to be 68.95±5.62 and 59.26±5.63, respectively after incubation for 4 h, where as clarithromycin plain drug was inhibited *H. pylori* growth completely within 4 h of incubation. At the end of 8 h of incubation the bacterial growth of formulation batches (FBC2 and FBC4) was reduced to 65.12±6.28% and 58.32±5.24%, respectively (Fig. 7). Clarithromycin is one of the most active and predictable antimicrobial agents against *H. pylori* and also has highest eradication rate in monotherapy *in vivo*. Clarithromycin has MIC90 of 0.05 μg/ml against clinical isolates of *H. pylori* and is more active than erythromycin, roxithromycin, and azithromycin.

The clarithromycin formulations achieved complete growth inhibition only after 12 h of incubation. Continued incubation of *H. pylori* whole cells for up to 24 h in the presence of clarithromycin formulations completely inhibited the bacterial growth. This is due to the controlled delivery of clarithromycin beads meant that the microorganisms were exposed to less of the drug. Thus, the time required for complete inhibition was less for clarithromycin plain drug than for clarithromycin formulations because of the direct exposure of the drug to the *H. pylori*. The results clearly indicate

Table 3. *In Vitro* Drug Release Kinetic of Prepared Floating Beads of Clarithromycin

Batch code	Drug release kinetic, correlation coefficients (r^2)[a]			Release exponent (n)[b]
	Zero order	First order	Higuchi	
FBC1	0.9407	0.8957	0.9864	0.6274
FBC2	0.9615	0.8649	0.9895	0.6951
FBC3	0.968	0.8548	0.9956	0.6852
FBC4	0.9750	0.8779	0.9851	0.6945
FBC5	0.9251	0.9151	0.9945	0.5489
FBC6	0.9342	0.9031	0.9845	0.5689
FBC7	0.9660	0.8654	0.9792	0.7356
FBC8	0.9383	0.8783	0.9745	0.6532
FBC9	0.9489	0.8608	0.9816	0.6885
FBC10	0.9298	0.8641	0.9918	0.6819
FBC11	0.9231	0.8798	0.9793	0.5919

a) By regression analysis. *b*) By following Peppas equation.

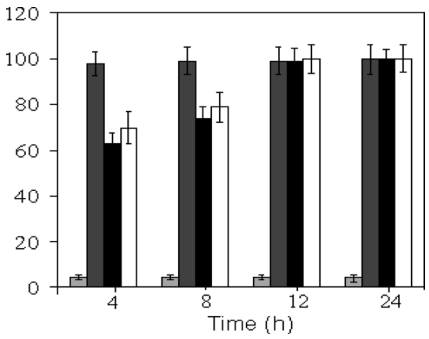

Fig. 7. Percentage of *H. pylori* Growth Inhibition

□ Placebo beads, ▩ clarithromycin plain, □ FBC2, ■ FBC4. Bars represent mean±S.D. (n=3).

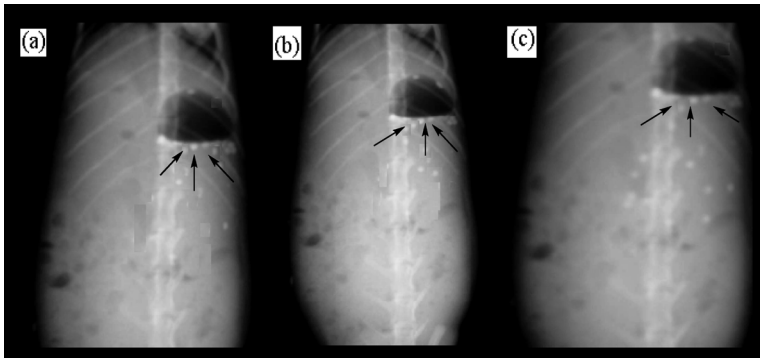

Fig. 8. X-Ray Photographs of Floating Beads of Clarithromycin in the Gastric Region of Rabbit after Dosing of Formulations in the Fasted State: (a) 1 h after Dosing, (b) 4 h after Dosing, (c) 6 h after Dosing

Arrows are indicating the floating beads in rabbit stomach.

that the beads have good %GI in *in vitro* culture and the clarithromycin floating beads may effectively target on the *H. pylori* surface and eradicate the *H. pylori* from the stomach. Thus, 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.

***In Vivo* Floating Efficiency** The X-ray photographs results of *in vivo* of floating efficacy of beads at different time intervals in rabbits stomach are shown in Figs. 8a—c. One hour after dosing, the beads showed good floatability (*ca.* 80%); 4 and 6 h after dosing about 60 and 50% of beads were found to be buoyant on gastric content, respectively, whereas the remaining beads were observed in a lower part of the stomach (Figs. 8b, c). The results clearly indicate that the prepared floating beads of clarithromycin remained buoyant for at least 6 h in rabbit's stomach and that they had good floatability *in vivo*.

Stability Studies of Formulations In view of the potential utility of formulation FBC2 for delivering clarithromycin to the stomach site for long term use, the stability studies were performed for this batch at 40 °C/75% RH for 6 months (climatic zone IV conditions for accelerating testing) to assess their long-term stability (2 years). The protocol conformed to the recommended World Health Organization document for stability testing of products intended for the global market.[35] After storage, formulation FBC2 was studied for physical appearance, beads size, beads shape (Fig. 1c), drug content and *in vitro* drug release characteristics. *In vitro* release data (Table 4) were analyzed for dissolution efficiency. No significant difference ($p>0.05$) in drug release characteristics was found in before and after the storage of batch FBC2 (Table 4). There was an insignificant change in the beads size distribution and shape (Fig. 1c), indicating that formulation FBC2 could provide a minimum shelf life of 2 years.

Table 4. Percentage of Clarithromycin Released from the Floating Beads (FBC2) before and after Storage at 40 °C/75% RH for 6 Months[a)]

Time (h)	Percentage of drug released	
	Before storage	After storage
0	0	0
0.5	20.23±0.98	17.36±1.25
1.0	31.41±1.89	27.32±1.56
2.0	41.23±1.49	43.25±1.98
3.0	54.40±1.92	51.49±1.95
4.0	63.25±2.13	60.68±2.13
5.0	71.27±2.31	68.62±2.32
6.0	76.32±2.43	74.36±2.45
7.0	82.51±2.24	78.28±2.31
8.0	87.23±2.19	84.65±2.65

a) Values indicate mean±S.D. ($n=3$).

Conclusion

In conclusion, the prepared gellan beads of clarithromycin has floated in SGF pH 2.0 for prolonged time of period and sustaining the drug release from the beads over the period of at least 8 h. The *in vitro H. pylori* inhibition study showed the good antimicrobial activity for formulated beads in *in vitro H. pylori* growth culture. Furthermore, X-ray study has shown that the floating beads have good floating efficiency in *in vivo* for long time in rabbit stomach and stability studies of prepared formulation viewed of the potential utility of formulation for long term usage. Thus, from the results we concluded that the gellan based floating beads containing clarithromycin appears to have promising potential for delivering clarithromycin at stomach site and may be very useful for targeting the clarithromycin at the site of infection and also for *H. pylori* eradication.

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