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# Floating In Situ Gelling System of Acetohydroxamic Acid for Clearance of *H. pylori*

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**The objective of this study was to develop a novel floating in situ gel system for sustained drug delivery of acetohydroxamic acid (FIGA) for eradication of *Helicobacter pylori (H. pylori)*. The FIGA was prepared by dissolving the different concentration of gellan in deionized water at 80°C. Different concentration of drug and calcium carbonate as floating agents were dispersed with stirring. In vitro results revealed that in situ gelling formulation forms rigid gels instantaneously and floated for longed period time of time in SGF pH 1.2. The formulation parameters, such as concentration of polymer, concentration of calcium carbonate, and concentration of drug, affected the in vitro drug release characteristic significantly. Absence of drug-polymer interaction was confirmed by differential scanning calorimetry analysis. The in vivo *H. pylori* clearance efficacy of prepared FIGA in reference to acetohydroxamic acid suspension following repeated oral administration to *H. pylori*-infected Mongolian gerbils was examined by microbial culture method. FIGA showed a significant anti-*H. pylori* effect in the in vivo gerbil model. It was noted that the required amount of acetohydroxamic acid for eradication of *H. pylori* was very less in FIGA than in the corresponding acetohydroxamic acid suspension. From the above results, it was concluded that the floating in situ gelling system has feasibility for forming rigid gels in the stomach and eradicated *H. pylori* from the gastrointestinal tract more effectively than acetohydroxamic acid suspension because of the prolonged gastrointestinal residence time of the formulation.**

**Keywords** floating in situ gelling; drug delivery; acetohydroxamic acid; in situ gelling system; stomach site-specific delivery; gellan

## INTRODUCTION

*Helicobacter pylori* is a gram negative bacteria that colonizes on human stomach, causes antral gastritis, and is frequently associated with peptic ulcer disease (Marshall & Warren, 1984). *H. pylori* is one of the world's most successful pathogens, infecting greater than 50% of the earth's population and also recognized as a risk factor for the development of gastric adenocarcinoma (Axon, 1994). Urease is essential for the motility and adhesion of *H. pylori* colonizing the gastric mucous layer (Nagahara et al., 1998). *H. pylori* produces high amounts of urease enzyme, which breaks down urea into ammonia and carbon dioxide and provides an acid-neutralizing cloud of ammonia that could protect the bacterium from the gastric acidity (Labigne & de Rease, 1996). Although *H. pylori* is susceptible to many antimicrobial agents in vitro, clinical trials with single antibiotics resulted in a low eradication rate of *H. pylori* (Labenz, 2001). This is because of the low concentration of the antibiotic reaching the bacteria under the mucosa, instability of drug in low pH of gastric fluid, and short residence time of the antibiotics in the stomach (Shah, Qagish, Patel, & Amiji, 1999). Therefore, combination of more than one antibiotic and an anti-secretory agent are required for the complete eradication of *H. pylori* (Suleymanlar et al., 1999).

Triple therapy, consisting of combined use of antibiotics, such as amoxicillin and clarithromycin/metronidazole, and a proton pump inhibitor, such as omeprazole, gives a high eradication rate, and is now applied for clinical treatment of *H. pylori* infection. However, eradication is not always successful due to some of the harmful side effects and cost of therapy of these drugs that may be encountered (Kawabami, Ogata, & Portorreal, 2001; Lin, Hsu, & Lai, 2002). Moreover, the resistance of *H. pylori* to antibiotics, including clarithromycin and metronidazole, has become a serious problem that may disturb treatment efficacy (Ohta et al., 2001). Other than the multi-antibiotic therapy, different therapeutic strategies have been examined to completely eradicate *H. pylori* from the stomach. One of these approaches is the use of non-antibiotics for *H. pylori* therapy. Since antibiotic-resistant strains of *H. pylori* have become a serious problem, non-antibiotic, urease inhibitors may be very useful to control *H. pylori*-associated gastroduodenal disease. AHA is a small molecule (molecular weight 75.07) that can permeate intact bacterial cells and effectively inhibit the urease activity of *H. pylori* (El Nujumi, 1991; Phadnis et al., 1996).

Stomach site-specific delivery of non-antibiotics has been suggested as an approach to improve the efficiency of therapy

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by increasing the gastric residence time, decreasing the diffusional distance, allowing more of the antibiotic to penetrate through the gastric mucus layer, and acting locally at the infectious site (Umamaheshwari, Jain, & Jain, 2003; Umamaheshwari, Suman, & Jain, 2004). By increasing the local concentration and contact time, stomach-specific delivery system also may minimize the resistance problems associated with administration of antibiotics (Labenz, 2001; Yokel, Dickey, & Goldberg, 1995). In our previous study (Rajinikanth, Balasubramanium, & Mishra, 2007) we described the development of a floating in situ gelling system of amoxicillin and evaluated the in-vivo efficiency in gerbils model. To overcome the constraints in the *H. pylori* treatment, with this optimistic approach, we have developed a novel floating in situ gelling system of AHA for the treatment of *H. pylori*-caused peptic ulcer diseases.

The prepared floating in situ gelling drug delivery systems are, in principle, capable of releasing drug in a sustained manner at the site of infection where *H. pylori* resides. Gellan gun is a bacterial anionic deacetylated polysaccharide secreted by *Pseudomonas elodea*. It has a characteristic gelling property, which is temperature and ionic dependant (Miyazaki, Kawasaki, Kudo, & Attwood, 2001). The proposed new gellan-based acetohydroxamic acid floating in situ gelling system has the advantages of having an ease of administration as a liquid and being more patient compliant, with the added advantage of having a simple manufacturing procedure. The calcium carbonate present in the formulation releases carbon dioxide in the gastric environment, thereby making the formulation buoyant, thus prolonging the residence time.

## MATERIALS

Acetohydroxamic acid was purchased from Sigma-Aldrich Chemical Ltd. (New Delhi, India) and Gellan gum (Gelrite®) was purchased from CP Kelco Company (Santiago, California, USA). Modified Skirrow's medium, Brucella broth, and fetal calf serum (FCS) were purchased from Himedia (Mumbai, India). Agarose was purchased from FMC BioProducts (Rockland, USA). All other reagents were of analytical grade.

## METHODS

### Preparation of Floating in Situ Gelling Solution

Gellan gum, at solution concentrations of 0.25–1.0% (w/v) were prepared in deionized water containing sodium citrate (0.25% w/v). Low level of cations present in the solution was sufficient to hold the molecular chains together and inhibit hydration. The gellan gum solutions were heated to 90°C with stirring. After cooling below 40°C, various concentrations of calcium carbonate and drug were added and dispersed well with continuous stirring. The resulting gellan in situ gel solution containing AHA was finally stored in amber colour narrow-mouth bottles until further use.

### Determination of Drug Content and Density of in Situ Gelling System

The drug content of the formulation was determined by dissolving 1 ml of in situ gelling formulation containing AHA in 100 ml of phosphate buffer of pH 7.4 followed by sonication for 30 min. The resulting solution was filtered through 0.4 μm syringe filter and the drug content of solution was measured at maximum absorbance at 503 nm using UV-Visible Spectrophotometer. The relative density of formulation was measured by mass by volume method. All the experiments were conducted in triplicate.

### Measurement of Viscosity of in Situ Gelling System

Viscosity determinations of the prepared formulations were carried out on a cone (0.8°) and plate geometry viscometer (Brookfield, USA) using spindle cp 40. Viscosity of ample solutions was measured at different angular velocities at a temperature of 37 ± 1°C. A typical run comprised changing the angular velocity from 0.5 to 100 rpm at a controlled ramp speed. After 6 sec at 0.5 rpm, the velocity was increased to 100 rpm with a similar wait at each speed. The hierarchy of angular velocity was reversed (100 rpm to 0.5 rpm) with a similar wait of 6 sec. The average of two readings was used to calculate the viscosity. Evaluations were conducted in triplicate.

### In Vitro Gelation Study

The gelation studies were carried out as described previously (Rajinikanth et al., 2007). The gelation cells were fabricated locally using Teflon®. The cells were cylindrical reservoirs capable of holding 3 ml of the gelation solution (simulated gastric fluid [SGF] of pH 1.2, without enzymes) .Within the cells, located at the bottom, was a 250-μl transparent plastic cup to hold the gel sample in place after its formation. Then, 100-μl of the preparation was carefully placed into the cavity of the cup using micropipette, and 2 ml of the gelation solution (SGF) was added slowly in reservoir. Gelation was observed by visual examination.

### In Vitro Floating Study

The in vitro floating study was determined using USP dissolution apparatus II having 500 ml of simulated gastric fluid (SGF, pH 1.2). The medium temperature was kept at 37°C. 10 ml prepared in situ gel formulations were drawn up using disposable syringe and placed into the Petri dish (4.5 mm internal diameter) and finally Petri dish containing formulation was kept in the dissolution vessel containing medium without much disturbance. The time the formulation took to emerge on the medium surface (floating lag time) and the time the formulation constantly floated on the dissolution medium surface (duration of floating) were noted.

### Measurement of in Vitro Drug Release

The release of AHA from the in situ gel preparations was determined as described previously (Rajinikanth et al., 2007) using USP dissolution test apparatus (USP 24) with a paddle stirrer at 50 rpm. This speed was slow enough to avoid the breaking of gelled formulation and was maintained with the mild agitation conditions believed to exist in vivo. The dissolution medium used was 500 ml of 0.1N HCl (pH 1.2), and temperature was maintained at 37°C. 10 ml formulation was drawn up using disposable syringe, the needle was wiped clean, and excess formulations removed from the needle end. The syringe end was then placed into the Petri dish (4.5 mm internal diameter) and the syringe plunger depressed slowly to extrude 10 ml, and finally Petri dish containing formulation was kept in the dissolution vessel containing dissolution medium without much disturbance. At each time interval, a precisely measured sample of the dissolution medium was removed and replenished with prewarmed (37°C) fresh medium. Absorbance of AHA in withdrawn samples was measured at 503 nm using UV-Visible Spectrophotometer. Interference from the excipients was negligible. Each study was conducted in triplicate for 8 hours.

### Drug-Polymer Interaction Studies

The physical state of the drug in the samples was determined by DSC (Modulated DSC, Q 1000 TA equipped with software Pyris 6.0). Samples of 3 mg of placebo mixture and drug-polymer physical mixture (AHA: gellan, 1:1 ratio at 40ºC/75% RH condition for 1 month) were placed in aluminum pans and then hermetically sealed with aluminum lids. The thermograms of samples were obtained at a scanning rate of 5ºC/min over a temperature range of 40 to 150°C under inert atmosphere flushed with nitrogen at the rate of 20 ml/min. All tests were performed twice.

### In Vivo *H. pylori* Clearance Study

The bacterial strain used in this study was originally isolated from a human patient (age 50 years) with gastric ulcer in SSG hospital, Banaras Hindu University, Varanasi, India. Six week old male specific pathogen free Mongolian gerbils (Body weight 50–60 g) were purchased from Central Drug Research Institute (Lucknow, India) and were maintained under standard laboratory conditions (room temperature, 23° ± 2°C; relative humidity, 55% ± 5%; 12/12 hours light/dark cycle) with free access to a commercial rodent diet and tap water. Six animals in each group were assigned to 9 groups and were inoculated with 1 mL culture broth via intragastric gavage after fasting for 24 hours. Each dose contained $10^9$ CFU of *H. pylori*. The protocols of the study was approved by the Institutional Animals Ethical Committee of the Department. Fourteen days after infection, acetohydroxamic acid was orally administered once a day for 3 consecutive days at a dose of 1, 4, 10, or 40 mg/kg in the form of either floating in situ gel or acetohydroxamic acid suspension (acetohydroxamic acid was dispersed well in 0.5% w/v of methylcellulose solution). Placebo floating in situ gel solution, used as a control, was administered in the same manner.

### Microbial Culture Method

One day 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/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, MD). The viable cell counts for each stomach were calculated by counting the number of colonies on the agar plates. The colonies were identified as *H. pylori* by morphology and urease activity (Nagahara et al., 1998).The number of colonies per plate was counted and expressed as log CFU per gastric wall. The advantage of this evaluation method is that errors caused by sampling site variation can be avoided because the whole stomach is used to determine the bacterial cell count.

### Statistical Analysis

The difference between the control-treated and the amoxicillin-treated groups in bacterial counts of gastric wall were statistically analyzed by one-way analysis of variance with post-test Dunnett's multiple comparison test. Statistically significant differences between groups were defined as $P < 0.05$.

## RESULT AND DISCUSSION

### Evaluation of Formulations

The composition and physico-chemical properties of the prepared formulations are shown in Table 1. The drug content uniformity, clarity, and pH of the formulations were found to be satisfactory and the formulations were liquid at room temperature. An ideal in situ gelling delivery system should be a free-flowing liquid with low viscosity under non-physiological conditions to allow reproducible administration into stomach condition. It also should undergo in situ phase transition to form a strong gel capable of withstanding shear forces in gastrointestinal tract and to sustain drug release under gastric environment. However, the nature of the gel formed depended upon the polymer and calcium carbonate concentration. The in situ formed gel should preserve its integrity without dissolving or eroding for prolonged period to facilitate sustained release of drugs locally.

The developed formulations met all prerequisites to become an in situ gelling floating system, gelled, and floated instantaneously in the pH conditions of the stomach. The relative density of the batches were measured and no significant differences

TABLE 1
Formulation Variables and Characterization of Floating in Situ Gelling Systems of Acetohydroxamic Acid

| Batches | Gellan (% w/v) | Calcium Carbonate (%w/v) | AHA (% w/v) | DCU (%)[a] | Gelation (in pH1.2) | Floating Lag Time (min) | Duration of Floating (h) |
|---|---|---|---|---|---|---|---|
| FIGA1 | 0.25 | 0.50 | 0.50 | 99.29 ± 0.89 | ++ | < 1 | > 20 |
| FIGA2 | 0.50 | 0.50 | 0.50 | 99.12 ± 0.56 | +++ | < 1 | > 20 |
| FIGA3 | 0.75 | 0.50 | 0.50 | 99.45 ± 0.79 | +++ | < 2 | > 20 |
| FIGA4 | 1.00 | 0.50 | 0.50 | 98.98 ± 0.92 | +++ | < 2 | > 20 |
| FIGA5 | 0.50 | 0 | 0.50 | 99.12 ± 0.12 | + | No | No |
| FIGA6 | 0.50 | 0.25 | 0.50 | 99.09 ± 0.32 | + | 10 | 2 |
| FIGA7 | 0.50 | 1.00 | 0.50 | 99.45 ± 0.65 | +++ | < 1 | > 20 |
| FIGA8 | 0.50 | 2.00 | 0.50 | 98.98 ± 0.28 | +++ | 10 | < 20 |
| FIGA9 | 0.50 | 0.50 | 0.25 | 99.01 ± 0.98 | ++ | < 1 | < 20 |
| FIGA10 | 0.50 | 0.50 | 1.00 | 98.56 ± 0.12 | +++ | < 1 | > 20 |
| FIGA11 | 0.50 | 0.50 | 2.00 | 99.08 ± 0.87 | +++ | < 1 | > 20 |

DCU: drug content uniformity.
[a]Percent of AHA present in the prepared in situ gelling systems, mean ± *SD* ($n$ = 3).
+ Gels after few minutes, dissolves rapidly; ++ Gelation immediate, remains for few hours; +++ Gelation immediate, remains for extended period.

between the batches were found. The relative density values of the batches were between 1.02 g/cm$^3$–1.035 g/cm$^3$. We have observed that in situ gelling solution of gellan concentration between 0.25%–1.0% is suitable for sustaining the AHA in SGF. The gellan concentration below 0.25% w/v was not able to sustain the AHA for long period of time; gellan concentration above 1.0% w/v forms gels at room temperature before administration due to high viscosity of solution. The photographs in Figures 1A and 1B show the formation of well-defined, irregular shaped, rigid gels in SGF pH 1.2. Upon contact with SGF pH 1.2, the in situ gelling formulation forms gels (Figure 1A), calcium carbonate creates carbon dioxide in acidic environment whose bubbles become entrapped into gel matrix of formulations, which causes formulations to float in the fluids for prolonged period of time (Figure 1B). The formulation prepared without calcium carbonate forms rigid gels in SGF and settled down at bottom of container as seen in Figure 1C.

## Viscosity of in Situ Gelling System

The rheological properties of the solutions are of importance in view of their proposed oral administration. In the selection of the concentration of gelling polymer a compromise is sought between a sufficiently high concentration for the formation of gels of satisfactory gel strength for use as a delivery vehicle, and a sufficiently low concentration to maintain an acceptable viscosity for ease of swallowing. Figures 2 and 3 shows the shear dependency on the viscosity of the AHA formulations. Measurements were performed under conditions representative of their proposed administration. All polymer concentrations showed evidence of shear thinning behavior, the effect being more pronounced at higher concentrations.

The solutions showed a marked increase in viscosity with increasing concentration of gellan as shown in Figure 2. The rheological properties of in situ gelling formulation of gellan at various levels of calcium carbonate for AHA is shown in Figure 3. The observed increase in viscosity with an increase in concentration of calcium carbonate has been noted previously for gellan (Moorehouse et al., 1981; Rajinikanth et al., 2007) and was attributed to a consequence of increasing chain interaction with polymer concentration. Increasing the calcium carbonate content in the formulation simultaneously increased the viscosity at all polymer concentrations studied. Since the calcium carbonate is present in the formulations as insoluble dispersion, an increase in its concentration proportionally increased the number of particles dispersed, thus contributing to the increased viscosity.

## Gelling Properties of in Situ Gelling System

The gelation study was conducted in 0.1N HCl, pH 1.2 (SGF). The mechanism of gelation involves the formation of double helical junction zones followed by aggregation of double helical segments to form a three-dimensional network by complexation with cations and hydrogen bonding with water (Grasdalen & Smidsroed, 1987). All the formulations showed instantaneous gelation when contacted with the SGF. Sol to gel transformation of gellan occurs in the presence of either monovalent or divalent cations in contact with the gastric fluids

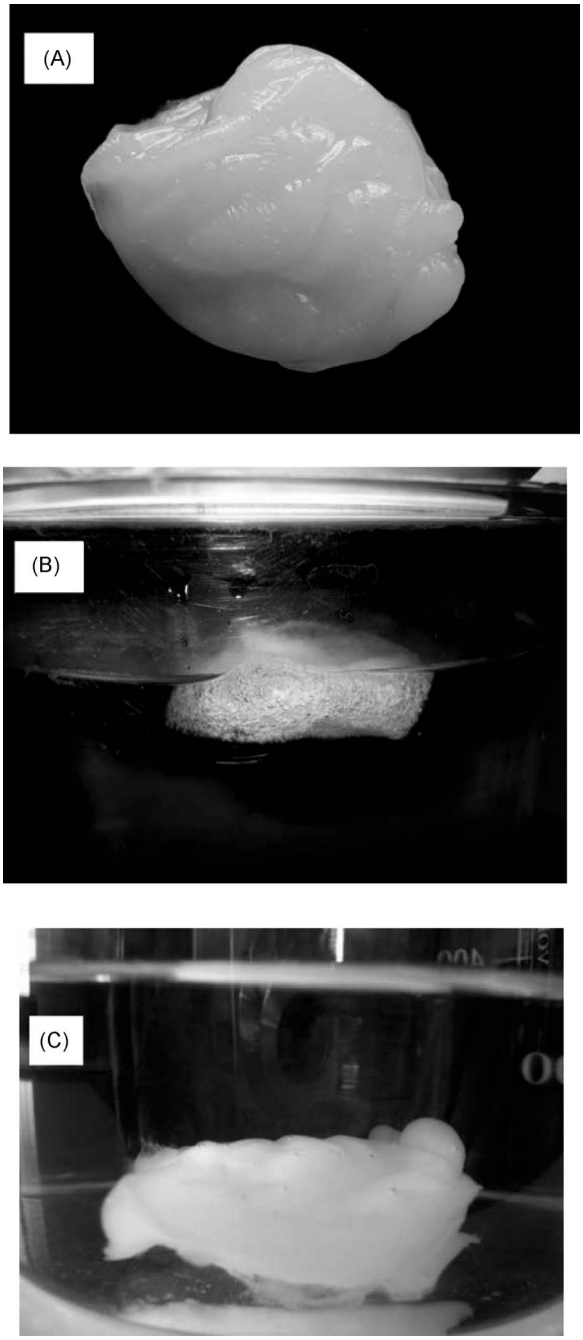


FIGURE 1. Photographs showing the appearance of gellan gels formed in SGF (pH 2.0); (A) Gellan gel, (B) Floating gellan gel in SGF pH 1.2, (C) Non-floating gellan gel in SGF pH 1.2.

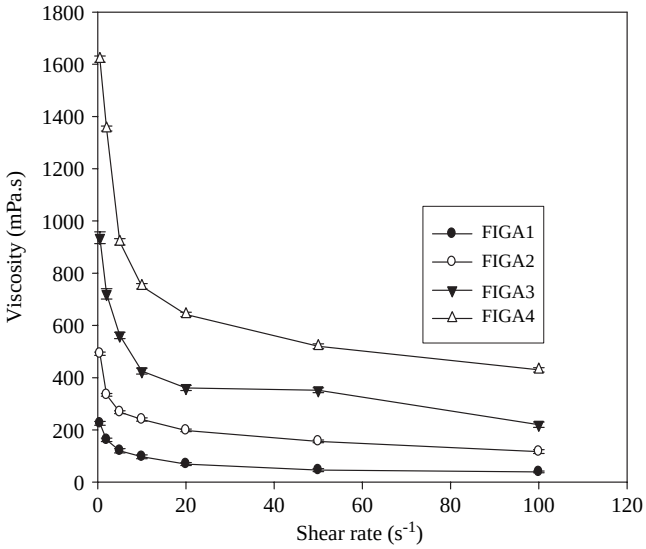


FIGURE 2. Effect of gellan concentrations on the viscosity of floating in situ gelling systems of acetohydroxamic acid at 0.5% w/v calcium carbonate concentration Bars represents ± *SD* (*n* = 3).

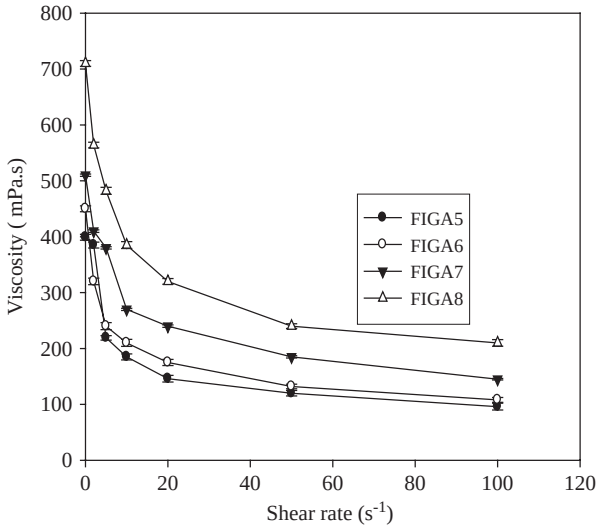


FIGURE 3. Effect of calcium carbonate concentrations on the viscosity of floating in situ gelling systems of acetohydroxamic acid 0.5% w/v gellan concentration. Bars represents ± *SD* (*n* = 3).

(Deshpande, Shah, Rhodes, & Malick, 1997). The calcium carbonate present in the formulation as insoluble dispersion is dissolved and releases carbon dioxide on reaction with acid, and the in situ releases calcium ions resulting in formation of gel with floating characteristics. It is established (Choi, Park, Hwang, & Park, 2002) that formulations containing calcium carbonate produce a significantly stronger gel than those containing sodium bicarbonate. This is due to the internal ionotropic gelation effect of calcium on gellan (Kedzierewicz et al., 1999).

The formulation containing calcium carbonate gelled more instaneously than that of the formulation not containing calcium carbonate (Table 1). This could be explained by the

fact that calcium carbonate is present in the formulation as insoluble dispersion and will only become soluble in the acidic medium and release calcium ions, which cause gelation of gellan. Gellan formulations with low content of calcium carbonate (less than 0.5% w/v) formed weak gels. Such vehicles are not suitable as oral liquid formulations, as they will be removed earlier from the stomach by the peristaltic movements. The formulation containing high concentration of calcium carbonate forms a rigid gel in a short gelation time of the delivery system in the stomach. In addition, the optimum level of polymer and calcium carbonate combinations demonstrated adequate gel strength when pressed with a pair of fine forceps, indicating that they will withstand the shear forces likely to be encountered in the stomach. Thus, such vehicles will have longer residence time than oral solutions. Ideally, an in situ gelling delivery system should be a free flowing liquid to allow reproducible oral administration as a liquid.

## Floating Properties of in Situ Gelling Systems

The buoyancy of the prepared formulations was performed in SGF pH 1.2. Both buoyancy lag time and buoyancy duration were in Table 1. Formulations containing calcium carbonate demonstrated excellent floating ability (Figure 1B), while formulations not containing this agent settled at the bottom of the medium (FIGA5) as seen in Figure 1C.

The in situ gelling formulation makes contact with an acidic medium and forms gel by cross linking with $Ca^{++}$ ions and forms a three-dimensional gel network in SGF. The calcium carbonate effervesced, releasing carbon dioxide and calcium ions. The released carbon dioxide is entrapped in the gel network, producing buoyant formulation; then, calcium ion reacted with gellan and produced a cross linked three dimensional gel network that might restrict the further diffusion of carbon dioxide and drug molecules and has resulted in extended period of floating and drug release, respectively (Chandrasekaran & Thailambad, 1990; Grasdalen & Smidsroed, 1987). The floating ability of the formulation mainly depends on calcium carbonate and gellan concentrations. The lowest level of calcium carbonate that produced a buoyant gel system for the duration of drug release study was found to be 0.5% (w/v) at all polymer levels. The formulation with more 0.5% w/v concentrations of calcium carbonate and above remained buoyant after the buoyancy test period (> 20 h) and the buoyancy was not influenced by the amounts of drug added to the formulations. At 0.25% w/v calcium carbonate, the gel was not able to retain the released carbon dioxide in the gel network.

Increasing the calcium carbonate concentration, the floating lag time was reduced and the duration of floating was increased, as shown in Table 1. The increase in the amount of $Ca^{++}$ and $CO_2$ content at increased calcium carbonate concentration are responsible for the observed reduction in floating lag time and increased duration of floating. Similarly, an increase in the polymer concentration resulted in decreased floating lag time and an increase in floating duration of the prepared systems (Singh & Kim, 2000). Various drug loading did not produce any significant change in floating properties.

## In Vitro Drug Release

In vitro drug release studies of prepared floating in situ gels were performed in SGF pH 1.2 for 8 h. Figure 4 illustrates the effect of gellan concentration on in vitro drug release. With increase in gellan polymer concentration, a significant ($p < 0.01$) decrease in the rate and extent of drug release was observed. This is due to increasing the path length through which the drug molecule has to diffuse, and the time required to traverse the gel

TABLE 2
In Vitro Release Kinetic Parameters of AHA from Floating in Situ Gelling Systems of Gellan

| | Zero-Order | | First-Order | | Higuchi | | Peppas Model | |
|---|---|---|---|---|---|---|---|---|
| Batch Code | K (mg/h) | *r* | K ($h^{-1}$) | *r* | K ($mg/h^{1/2}$) | *r* | n | $r^2$ |
| FIGA1 | 0.8122 | 0.8720 | 0.8653 | 0.9546 | 0.8325 | 0.9823 | 0.3945 | 0.9985 |
| FIGA2 | 0.7039 | 0.8840 | 0.6562 | 0.9696 | 0.6235 | 0.9890 | 0.4125 | 0.9965 |
| FIGA3 | 0.5203 | 0.8949 | 0.6023 | 0.9641 | 0.5245 | 0.9983 | 0.4532 | 0.9758 |
| FIGA4 | 0.5781 | 0.9137 | 0.5123 | 0.9751 | 0.5125 | 0.9918 | 0.4568 | 0.9878 |
| FIGA5 | 0.8178 | 0.8040 | 0.8523 | 0.9461 | 0.8450 | 0.9873 | 0.3546 | 0.9876 |
| FIGA6 | 0.6063 | 0.8795 | 0.5623 | 0.9335 | 0.7211 | 0.9893 | 0.4125 | 0.9956 |
| FIGA7 | 0.7254 | 0.9468 | 5.6232 | 0.9853 | 0.5456 | 0.9901 | 0.4312 | 0.9678 |
| FIGA8 | 0.6569 | 0.9624 | 0.4356 | 0.9889 | 0.4768 | 0.9964 | 0.5232 | 0.9898 |
| FIGA9 | 0.6766 | 0.9666 | 0.6321 | 0.9654 | 0.6256 | 0.9897 | 0.3842 | 0.9798 |
| FIGA10 | 0.6995 | 0.9136 | 0.6343 | 0.9542 | 0.6325 | 0.9912 | 0.4012 | 0.9985 |
| FIGA11 | 0.7253 | 0.9069 | 0.6245 | 0.9635 | 0.6412 | 0.9968 | 0.3978 | 0.9984 |

K: release rate constant; *r*: coefficient of correlation; n: release exponent; $r^2$: coefficient of determination.

TABLE 3
Effect of Repetitive Administration of Acetohydroxamic Acid Suspension and Acetohydroxamic Acid Floating in Situ Gel Formulations against Gastric Infection Caused by *H. pylori* in Mongolian Gerbils

| Formulations | Dose[a] (mg/kg) | Clearance Rate (No. of Gerbils Cleared Infection) /Total No. (%) | Bacterial Recovery (Log CFU/Stomach)[b] |
|---|---|---|---|
| Placebo FIG (Control) | 0 | 0/6(0) | 7.76 ± 0.16 |
| Acetohydroxamic acid suspensions | | | |
| | 1 | 0/6 (0) | 7.54 ± 0.38 |
| | 4 | 1/6 (0) | 6.48 ± 0.82 |
| | 10 | 2/6 (33) | 4.52 ± 0.78 |
| | 40 | 4/6 (66) | 5.09 ± 0.51* |
| Acetohydroxamic acid floating in situ gel formulations (AFGA4) | | | |
| FIGA4 | 1 | 2/6 (33) | 6.32 ± 0.29 |
| FIGA4 | 4 | 4/6 (66) | 4.98 ± 0.68* |
| FIGA4 | 10 | 5/6 (83) | 3.12 ± 0.72** |
| FIGA4 | 40 | 6/6 (100) | ND |

FIG: floating in-situ gel; CFU: colony-forming unit; ND: not detected.
[a]Drugs were administered once daily for 3 consecutive days.
[b]Bacterial cell counts less than $10^{1.45}$ CFU were considered to be $10^{1.45}$ to calculate the mean. Values are means ± *SE*.
*$P < 0.05$,
**$P < 0.01$ (both significant level in reference to control).

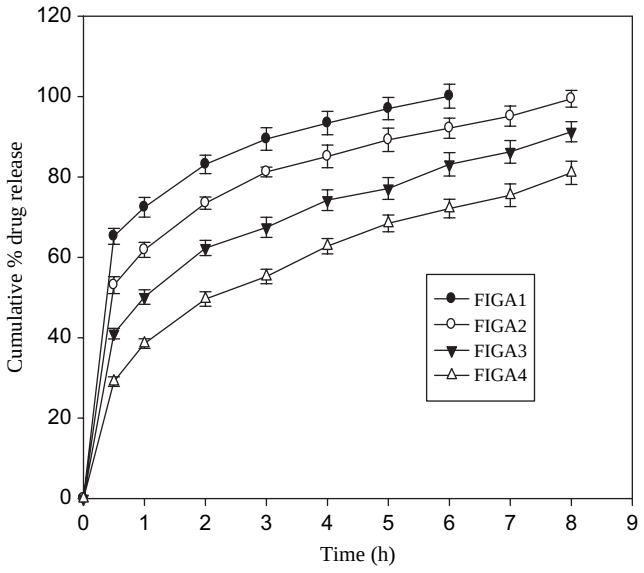


FIGURE 4. Effect of polymer concentration on in vitro drug release from floating in situ gelling system of AHA. Bars represent mean ± *SD* ($n = 3$).

matrix is increased, causing reduction in rate and extent of drug release at higher gellan concentrations. The release of drug from these gels was characterized by an initial phase of rapid release (burst effect) and followed by a second phase of moderate release; as gelation proceeds, the remaining drug was released at a slower rate. The initial burst release helps to enhance the AHA concentration for the effective *H. pylori* clearance shortly after oral administration of formulation. This biphasic pattern of release is a characteristic feature of matrix diffusion kinetics (Rajinikanth, Sankar, & Mishra, 2003). The initial fast release of AHA from the prepared systems could be explained by the fact that these systems were formulated in an aqueous vehicle, the matrix formed on gelation was already hydrated, and drug dissolves rapidly before formation a rigid gel in medium. However, the results showed that the formed gels had the ability to retain AHA for the duration of the study (8 h). The initial burst effect was considerably reduced with increase in polymer concentration. In formulations containing lower concentration of gellan (FIGA1) about 60% of the drug was released into the medium after 0.5 h, and then the drug was gradually released to the extent of 90% in 3h (Table 1 & Figure 4). In the case of formulation FIGA3 and FIGA4, approximately 40% and 30% of drug released in 0.5 h, respectively, and remaining drug was gradually released up to 8 h (Table 1 & Figure 4). These results suggest that in situ gelling formulation with less than 0.5% w/v gellan was not able to retain drug for the duration of the study (8 h) and not suitable for sustaining the water soluble drugs like AHA.

Figure 5 demonstrate the effect of different calcium carbonate concentration on in vitro release from in situ gels of gellan. In vitro drug release profiles of formulation with different amount

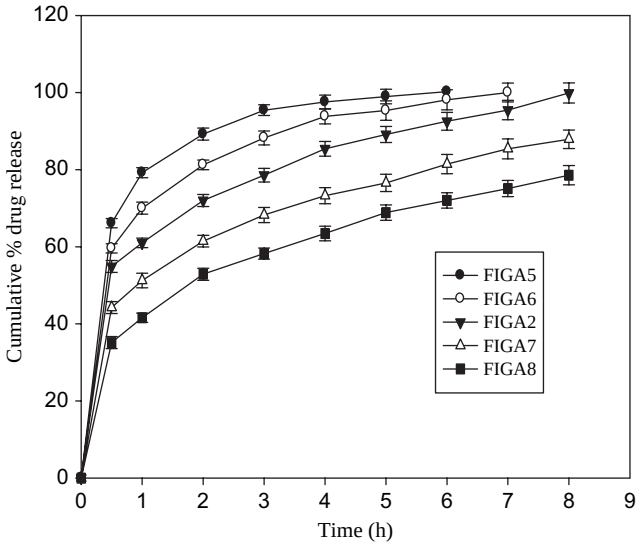


FIGURE 5. Effect of calcium carbonate concentration on in vitro drug release from floating in situ gelling system of AHA. Bars represent mean ± *SD* (*n* = 3).

of calcium carbonate (batches FIGA6 to FIGA8) was compared with control formulation (formulation without calcium carbonate, FIGA5). Percentage drug release was always higher for control formulations than calcium carbonate containing formulations (Figure 4). In control formulation, the gelation was due the presence of monovalent cations, $Na^+$ and $H^+$ present in the formulation and dissolution medium, respectively, whereas in calcium carbonate containing formulations, the gelation was predominantly by divalent cation $Ca^{++}$. With increase in calcium carbonate concentration in formulations, decreased percentage of drug release was observed. In the absence of calcium ions, the sodium ions present in the formulation will be predominantly in ionized form and cause weak gelation. Hence, the release rate was always higher for formulations without calcium carbonate than for the formulations containing calcium carbonate (Chandrasekaran & Thailambad, 1990; Deasy & Quigley, 1991). Effect of drug loading on in vitro drug release from in situ gel formulation are shown in Figure 6. The results indicate that there was no significant difference ($P > 0.01$) in the rate and extent of drug release from the floating in situ gels containing different concentrations of AHA . In order to investigate the drug release mechanism, the release data were fitted to models representing zero order, first order, and Higuchi's square root of time.

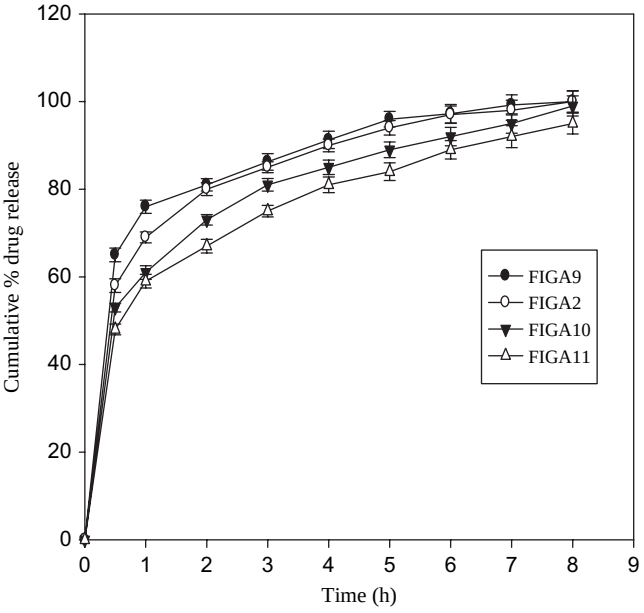


FIGURE 6. Effect of drug concentration on in vitro drug release from floating in situ gelling system of AHA. Bars represent mean ± *SD* (*n* = 3).

The linear regression analysis are summarized in Table 2. The in vitro release data from gels were analyzed according to the treatment proposed by Higuchi (1962) for drug release from semisolid vehicles containing dissolved drug. For the initial 50–60% release the cumulative amount Q of drug release per unit surface area from gels of initial drug concentration $C_0$ is proportional to the square root of time t:

$$Q = 2C_0\left(\frac{Dt}{\Pi}\right)^{1/2} \quad (1)$$

Figures 7 and 8 show the percent drug released versus square root of time. It is notable that the $r^2$ values of the linear regressions were greater than 0.98 and the residuals were randomly distributed for all the formulations studied, indicating that the data fit the Higuchi model well.

In order to investigate the drug release, data were fitted with models representing zero order, first order, and Higuchi model. The examination of coefficient of determination ($r^2$) values for different formulations indicated that drug release followed diffusion control mechanism from the FIGA. To explore the kinetic behavior, in vitro release results were further fitted into the following Peppas equation (Korsmeyer et al., 1983). $Mt/M\infty = Kt^n$, where $Mt/M\infty$ is the fraction of drug released after time t, K is a kinetic constant, and n is a release exponent. Values for coefficient of determinations, $r^2$, and the release exponent, n, are listed in Table 2. The values for n were in the range of 0.3945–0.5232, which characterizes the drug transport to occur by the Fickian drug diffusion mechanism.

## Drug-Polymer Interaction Studies

In an effort to investigate the possible physical interactions between drug and polymer, we have analyzed: (A) pure actohydroxaic acid, (B) Gellan polymer, and (C) physical mixtures of drug and gellan (1:1 ratio) using modulated DSC analysis

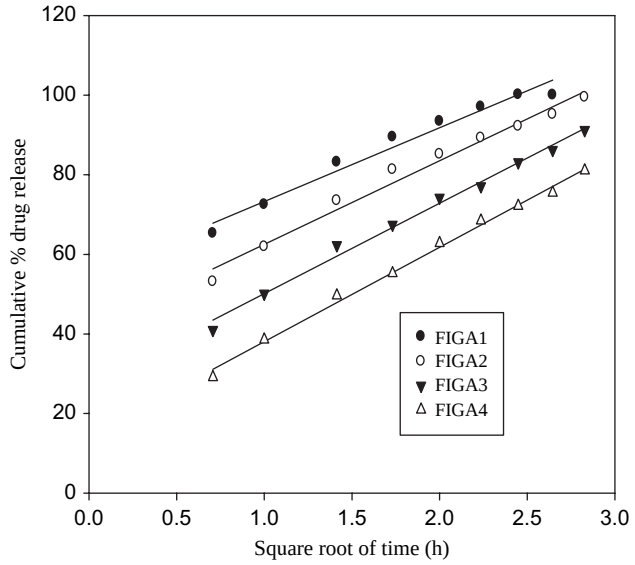


FIGURE 7. Higuchi Square root of time plot for floating in situ gelling system of AHA conatining diffrent polymer concentrations.

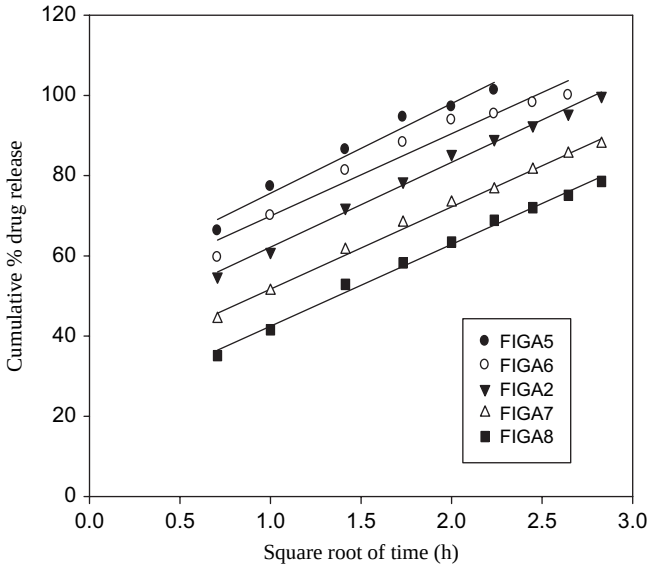


FIGURE 8. Higuchi Square root of time plot for floating in situ gelling system of AHA conatining calcium carbonate polymer concentrations.

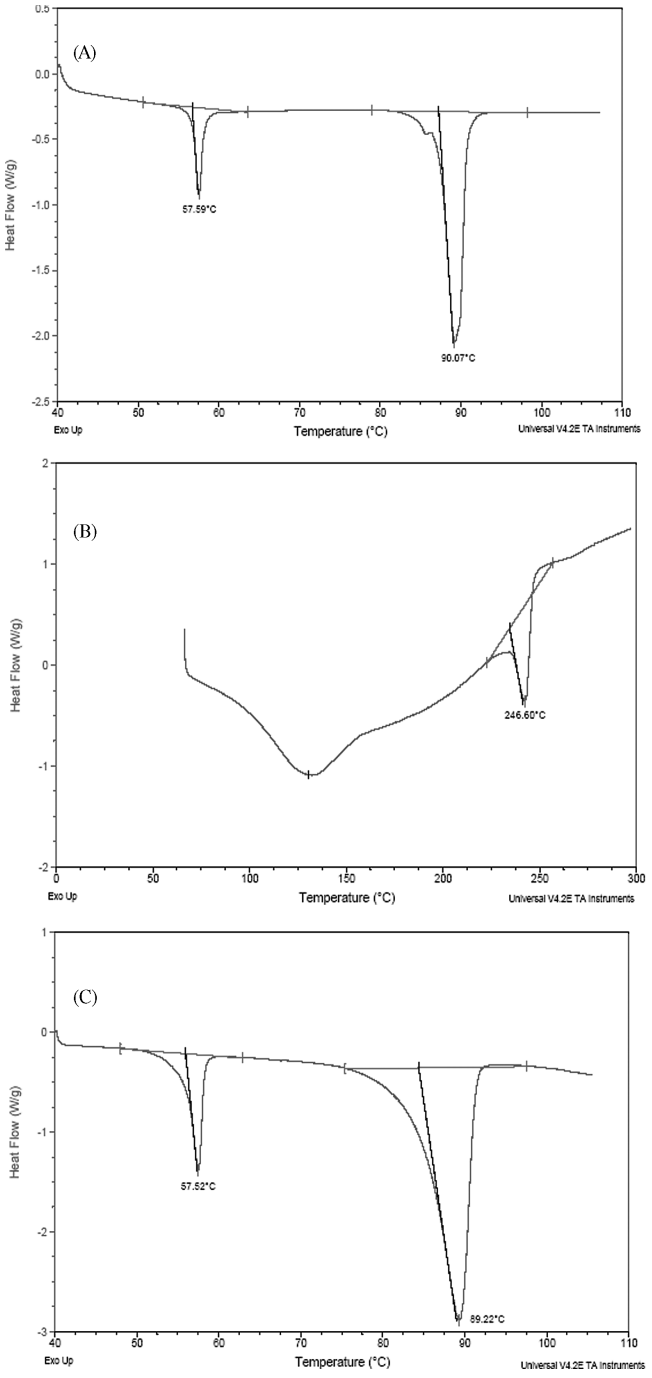


FIGURE 9. DSC theromograms of: (A) Acetohydroxamic acid pure; (B) Gellan polymer; and (C) physical mixtures of drug and polymer.

and the results are displayed in Figure 9. The DSC Thermogram shown a sharp endothermic peak at 90.07°C for pure acetohydroxamic acid due to the melting point of the drug (Figure 9A). The thermograms of gellan polymer and physical mixture of drug and polymer were given in Figures 9B and 9C, respectively. In gellan polymer, the endothermic peak, shown at 246.60°C, is attributed to the melting point of the gellan polymer. Drug-polymer mixture showed a broad small peak at 89.22°C, indicating the presence of drug in crystalline form. The broadening or reduction of sharpness of the endotherm peak is due to dilution of sample, which also appears at around 90°C in mixture. This result indicates there was no possible physical interaction between drug and polymer since there was no detectable shift of endotherm peak observed.

### In Vivo *H. pylori* Clearance Study

In vivo evaluation of in situ gelling systems of acetohydroxamic acid (FIGA) was carried out in Mongolian gerbils infected with human *H. pylori*. The in vivo clearance data of *H. pylori* after multiple administration of acetohydroxamic acid (AHA) in situ gel and the AHA suspension under fed conditions (acetohydroxamic acid doses at 1, 4, 10, and 40 mg/kg) are presented in Table 3. Since viable numbers of *H. pylori* have been reported to reach a plateau two weeks after bacteria inoculation (Hirayama, Takagi, Yokoyama, Iwao, & Ikeda, 1996), we therefore examined administration of AHA from two weeks after *H. pylori* inoculation. In the control group receiving no drug, around $10^{7.76 \pm 0.16}$ viable bacteria colonized the stomach. The mean bacterial count in gerbil's stomach after oral administration of the acetohydroxamic acid suspension decreased as the dose of AHA increased (Table 3); however, complete clearance of *H. pylori* was not obtained even with the highest dose (40 mg/kg) as shown in Table 3. This is because of the short residence time of AHA suspension in the stomach and the low concentration of AHA reaching the bacteria under the gastric mucus layer. The residence time of AHA in the stomach after oral administration of the conventional dosage form is expected to be short (Rajinikanth et al., 2007; Umamaheshwari et al., 2003). Therefore, the resulting insufficient duration of contact with the gastric mucosa by conventional dosage form of AHA may be the reason for the incomplete eradication of *H. pylori*. The mean bacterial count after 3 days of treatment with FIGA, with an AHA dose of 4.0 mg/kg ($10^{4.98 \pm 0.68}$), was almost equal to that of AHA suspension at the dose of 40.0 mg/kg ($10^{5.09 \pm 0.51}$), which is significantly ($p < 0.05$) lower than that of AHA suspension, which clearly indicates that due to stomach specific delivery of AHA from in situ gel, it is equally effective at much lower dose than the AHA suspension. On other hand, anti-*H. pylori* therapy with FIGA at AHA dose of 10 mg/kg, ~83% of the bacteria ($10^{3.12 \pm 0.72}$) were eradicated from gerbils' stomachs and no bacterial colony was detected at AHA dose of 40.0 mg/kg in the original dilution from all gastric sample from these groups, indicating 100% clearance rate (Table 3). This is because of the longer residence time of FIGA in the stomach, which enabled high concentration of AHA to reach the bacteria underlying the gastric mucosal layer.

In this study we confirmed almost complete *H. pylori* eradication using anti-urease drug (monotherapy as floating in situ gelling system). However, it also has been reported that treatment with antibiotics (by triple therapy) resulted in the acquisition by *H. pylori* of resistance to antibiotics, and side effects including diarrhea in patients (Wang et al., 2000). In fact, treatment with urease inhibitors such as AHA may be less toxic than the triple therapy and also may minimize the resistant problems, although a long-term toxicity test of these urease inhibitors in rodents is further required (Ohta et al., 2001). The urease activity in *H. pylori* might essentially contribute to the colonization ability. The data obtained in the present in vivo study using the urease inhibitor AHA in Mongolian gerbils, clearly supported the results of the in vitro experiments previously reported (Kodama et al., 2005; Ohta et al., 2001), and provided evidence that urease of *H. pylori* plays an important role in the colonization in the gastric mucosa. The results from the above studies indicated clearly that the AHA, in the form of floating in situ gelling system, eradicated *H. pylori* from the gerbils' stomachs more effectively than AHA in the form of suspension because of the prolonged gastrointestinal residence time attributed to increase the efficiency of the therapy. Floating in situ gelling systems form containing antibacterial agent like AHA should be useful for the complete eradication of *H. pylori*. Further, the dose of FIGA required to achieve total clearance was ~10 times less than that of the AHA suspension

## CONCLUSION

The prepared gellan-based floating in situ gelling of AHA has feasibility of forming gels in stomach and sustaining the drug release from the gels over the period of at least 8 h. This new floating in situ gel may be used as an alternative to conventional dosage forms by virtue of its ability to form a rigid gel and enhance local AHA concentration through its sustained drug release and longer residence time in the stomach. Also important is its ease of administration with reduced frequency of administration resulting in better patient acceptance. Further, the prepared in situ gels were effective in clearing *H. pylori* in infected gerbils' stomachs at a dose level that was comparatively very less than that of AHA suspension, which is important from the viewpoint of reducing adverse effect during the therapy. The floating in situ gelling system of gellan appears to offer a promising potential for delivering AHA at stomach site and may be used as a stomach site-specific delivery system of AHA for the treatment of peptic ulcer disease caused by *H. pylori*.

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