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Development and evaluation of a novel floating in situ gelling system of amoxicillin for eradication of *Helicobacter pylori*

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Abstract

The aim of this study was to develop a new intra-gastric floating in situ gelling system for controlled delivery of amoxicillin for the treatment of peptic ulcer disease caused by *Helicobacter pylori* (*H. pylori*). Gellan based amoxicillin floating in situ gelling systems (AFIG) were prepared by dissolving varying concentrations of gellan gum in deionized water containing sodium citrate, to which varying concentrations of drug and calcium carbonate, as gas-forming agent, was added and dissolved by stirring. The formulation variables like concentration of gellan gum and calcium carbonate significantly affected the in vitro drug release from the prepared AFIG. The in vivo *H. pylori* clearance efficacy of prepared AFIG in reference to amoxicillin suspension following repeated oral administration to *H. pylori* infected Mongolian gerbils was examined by polymerase chain reaction (PCR) technique and by a microbial culture method. AFIG showed a significant anti-*H. pylori* effect in the in vivo gerbil model. It was noted that the required amount of amoxicillin for eradication of *H. pylori* was 10 times less in AFIG than from the corresponding amoxicillin suspension. The results further substantiated that the prepared AFIG has feasibility of forming rigid gels in the gastric environment and eradicated *H. pylori* from the gastrointestinal tract more effectively than amoxicillin suspension because of the prolonged gastrointestinal residence time of the formulation.
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Keywords: Oral in situ gelling system; Controlled delivery; Amoxicillin; Gastric residence time; Floating drug delivery

1. Introduction

Helicobacter pylori (*H. pylori*) is one of the most common pathogenic bacterial infections, colonizing an estimated half of all humans (Marshall and Warren, 1984). It is associated with the development of serious gastro duodenal disease—including peptic ulcers, gastric lymphoma and acute chronic gastritis (Crescenzi et al., 1990). *H. pylori* reside mainly in the gastric mucosa or at the interface between the mucous layer and the epithelial cells of the antral region of the stomach (Peterson, 1991). The discovery of this microorganism has revolutionized the diagnosis and treatment of peptic ulcer disease. Most antibacterial agents have low minimum inhibitory concentrations (MIC) against *H. pylori* in culture. And also single antibiotic therapy is not effective for the eradication of *H. pylori* infection in vivo. This is because of the low concentration of the antibiotic reaching the bacteria under the mucosa, instability of the drug in the low pH of gastric fluid and short residence time of the antibiotic in the stomach (Shah et al., 1999). Combination of more than one antibiotic and anti-secretory agent are required for complete eradication of *H. pylori* but these regimens are not fully effective. Patient compliance, side effects and bacterial resistance are the other problems. Other than the multi-antibiotic therapy, different therapeutic strategies have been examined to completely eradicate *H. pylori* from the stomach.

One way to improve the efficacy in eradicating the infection is to deliver the antibiotic locally in the stomach (Yokel et al., 1995; Shah et al., 1999). Better stability and longer residence time will allow more of the antibiotic to penetrate through the gastric mucus layer to act on *H. pylori* (Umamaheshwari et al., 2004).

The reason for the incomplete eradication of *H. pylori* is probably due to short residence time of antimicrobial agents in the

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stomach so that effective antimicrobial concentration cannot be achieved in the gastric mucous layer or epithelial cell surfaces where *H. pylori* exists (Cooreman et al., 1993; Atherton et al., 1995). The other reason may be the degradation of antibiotics in gastric acid (Axon, 1994; Giacomo et al., 2001). Therefore, some researchers had prepared and reported new amoxicillin formulations, such as floating tablets, mucoadhesive tablets, pH sensitive excipients composition mucoadhesive microspheres, etc., which were able to reside in stomach for an extended period for more effective *H. pylori* eradication (Umamaheshwari et al., 2003; Hilton and Deasy, 1992). Access of antimicrobial drugs to the site is restricted from both the lumen of the stomach and the gastric blood supply. *H. pylori* may also have acquired resistance to the commonly used antimicrobial agents. As conventional drug delivery systems do not remain in the stomach for prolonged periods, they are unable to deliver the antibiotics to the site of infection in effective concentrations and in fully active forms. Therefore, it is necessary to design drug delivery systems that not only alleviate the shortcomings of conventional delivery vehicles but also deliver the antimicrobials to the infected cell lines. 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) (Katayama et al., 1999).

Keeping above facts in mind we made an attempt to develop a new floating in situ gelling system of amoxicillin using gellan as gelling polymer with a potential to use in treatment of *H. pylori* caused stomach ulcer. The proposed new gellan based amoxicillin floating in situ gelling systems (AFIG), would have the advantage of ease of administration, as being a liquid, and also be more patient compliant.

Amoxicillin is a semisynthetic, 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 (Suleymanlar et al., 1999; Vakil and Cutler, 1999). 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 et al., 1999, 2001). The basic strategy adopted in this study involved incorporation of calcium carbonate and sodium citrate in a gellan gum-amoxicillin dispersion. Initially, the calcium carbonate becomes soluble in the acidic environment of the stomach, and the released calcium ions then are complexed by the sodium citrate. However, a slow conversion of the complexed calcium into free calcium causes gelation of gellan, the gelled material floats upwards in the stomach, with a potential to release its drug over a period of time. The calcium carbonate present in the formulation, releases carbon dioxide in the gastric environment, thereby making the formulation buoyant, thus prolonging the residence time.

2. Materials and methods

2.1. Materials

Amoxicillin was gifted by Ranbaxy Laboratories 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) and Taq DNA polymerase was purchased from Takara Shuzo, Otsu, Shiga, Japan. All other reagents were of analytical grade.

2.2. Animals

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, $23 \pm 2\,^{\circ}C$; relative humidity, $55 \pm 5\%$; 12-h light:12-h dark cycle) with free access to a commercial rodent diet and tap water.

3. Methods

3.1. Preparation of 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 amoxicillin was finally stored in amber colour narrow mouth bottles until further use.

3.2. Measurement of viscosity of in situ gelling solutions

The viscosity of sols were determined by cone and plate viscometer with cone angle 1° 34 (TV-20H, model E. Tokimec Co., Tokyo, Japan) at 5 or 20 °C using 1 ml aliquot of sample. Viscosity measurement for each sample was done in triplicate, with each measurement taking approximately 30 s.

3.3. In vitro gelation study

The gelation studies were carried out as described previously (Zhidong et al., 2006) with slight modification. 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.

3.4. In vitro floating study

The in vitro floating study was determined using USP dissolution apparatus II having 500 ml of simulated gastric fluid (pH

1.2) .The medium temperature was kept at 37 °C. Ten millilitre 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.

3.5. Measurement of in vitro drug release

The release of amoxicillin from the in situ gel preparations was determined as described by Zatz and Woodford (1987) with some modification 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 maintaining with the mild agitation conditions believed to exist in vivo. The dissolution medium used was 500 ml of 0.1 N HCl (pH 1.2), and temperature was maintained at 37 °C. Ten millilitre 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 Petridish 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 amoxicillin in withdrawn samples was measured at 272 nm using UV Spectrophotometer (Shimadzu, UV-1601, Japan). Interference from the excipients was negligible. Each study was conducted in triplicate till 8 h.

3.6. 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 animals in each groups were assigned to nine groups and were inoculated with 1 ml culture broth via intra-gastric gavage after fasting for 24 h. Each dose contained 10^9 CFU of *H. pylori*. The protocols of the study was approved by Institutional Animals Ethical Committee of the Department. Fourteen days after infection, amoxicillin 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 amoxicillin suspension (amoxicillin was dispersed well in 0.5% (w/v) of methylcellulose solution). Placebo floating in situ gel solution, used as a control, were administered in the same manner.

3.7. 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 (Westblom et al., 1990).

3.8. Isolation of DNA

The DNA of *H. pylori* was isolated as described by Kusuhara et al. (1998). The gastric tissue was collected by centrifugation from 1 ml of homogenates from each gerbil stomach. The tissue was suspended in 500 μl of TE (Tris ethylene diamine tetra acetate, pH 8.0) containing100 mM NaCl and 100 g/ml egg white lysozyme, and incubated at room temperature for 10 min. Sodium dodecyl sulfate was added at a final concentration of 0.1%, and the mixture was incubated at 60 °C for 10 min. DNA was then extracted in an equal volume of phenol:chloroform (1:1), and precipitated at 20 °C for 2 h in the presence of 300 mM sodium acetate and two volumes of absolute ethanol. The precipitated DNA was collected by centrifugation and allowed to air dry. The pellet was then dissolved in 50 μl of TE.

3.9. Polymerase chain reaction (PCR) amplification of the 16S rRNA gene of H. pylori

The primers that recognize the *H. pylori*-specific region in the 16S rRNA gene have been described by Kusuhara et al. (1998) and Ho et al. (1991) with slight modification. The reaction mixture contained 5 μl of 10 × PCR buffer (500 mM KCl, 100 mM Tris–HCl, 15 mM MgCl2**),** 4 μl of deoxynucleotide solution (2.5 mM each dATP, dCTP, dGTP and dTTP), 0.5 μl of each primer (50 μM), 1.25 U Taq DNA polymerase and DNA in a final volume of 50 μl. DNA from the homogenates of each gerbil stomach was serially diluted 10-fold in sterile water, and 1 μl of each diluted sample was used in one assay. Each reaction tube was then placed in a thermal cycler (Perkin-Elmer 9600; Perkin-Elmer, Emeryville, CA, USA). The following conditions were used for the amplification, denaturation at 94 °C for 30 s; anealing at 56 °C for 30 s and extension at 72 °C for 1 min. A total of 36 cycles were performed, followed by an extension step at 72 °C for 7.0 min. The PCR product (501 bp) was separated by electrophoresis through a 2% gel containing ethidium bromide, and visualized with a UV light source. The PCR product from gel was photographed by alpha imager.

3.9.1. Statistics

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 $P < 0.05$.

4. Results and discussion

4.1. Evaluation of formulations

The composition of the developed formulations are shown in Table 1. The two main pre-requisites of in situ gelling systems are optimum viscosity and gelling capacity (speed and extent of gelation). The formulation should have an optimum viscosity that will allow easy swallowing as a liquid, which then undergoes a rapid sol–gel transition due to ionic interaction. Moreover, 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 bateche were measured and found to be no significant different between the bateches. The relative density values of the ateches are between 1.01 g/cm^3 (AFIG1) and 1.035 g/cm^3 (AFIG10).

Sol to gel transformation of gellan occurs in the presence of either monovalent or divalent cations in contact with the gastric fluids. The calcium carbonate present in the formulation as insoluble dispersion is dissolved and releases carbon dioxide on reaction with acid, and the in situ released calcium ions results in formation of gel with floating characteristics. 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 (in vivo) (Deshpande et al., 1997). It is established (Choi et al., 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).

4.2. Viscosity and gelling properties

The rheological properties of the solutions are of importance in view of their proposed oral administration. In the selection of the concentration of the 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. Figs. 1 and 2 show the shear dependency of the viscosity of the amoxicillin formulations. Measurements were performed under conditions representative of those of their proposed administration; the gellan solutions were maintained in the solution form by measurement at 20 °C. All polymer concentrations showed evidence of shear thinning behaviour, the effect being more pronounced at higher concentrations. The solutions showed a marked increase in viscosity with increasing concentration of gellan as shown in Fig. 1.

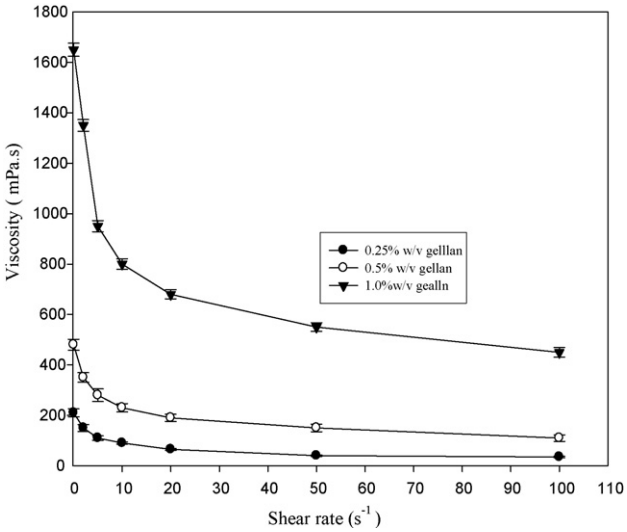

Fig. 1. Effect of the polymer concentration on the viscosity of floating in situ gelling system of amoxicillin at 0.5% (w/v) calcium carbonate concentration. Bars represent mean ± S.D. ($n = 3$).

Fig. 2 shows the rheological properties of gels of gellan at various levels of calcium carbonate for amoxicillin. The observed increase in viscosity with increase in concentration has been noted previously for gellan (Moorehouse et al., 1981) 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. Fig. 3 shows that there is no significant difference in viscosity

Table 1
Formulation variables and characterization of floating in situ gelling system of amoxicillin

Batches	Gellan(%, w/v)	Calcium carbonate (%, w/v)	Amoxicillin (%, w/v)	Gelation (pH 1.2)	Floating lag time (min)	Duration of Floating (h)
AFIG1	0.25	0.50	0.25	+++	<1	>24
AFIG2	0.50	0.50.	0.25	++	<1	>24
AFIG3	1.00	0.50	0.25	++	<2	>24
AFIG4	1.00	0	0.25	+	No	No
AFIG5	1.00	0.25	0.25	++	30	<1
AFIG6	1.00	1.00	0.25	+++	<1	>24
AFIG7	1.00	2.00	0.25	+++	<1	>24
AFIG8	1.00	0.50	0.5	++	<2	>24
AFIG9	1.00	0.50	1.0	+++	<1	>24
AFIG10	1.00	0.50	2.0	+++	<1	>24

(+) Gels after few minutes, dissolves rapidly; (++) gelation immediate, remains for few hours; (+++) gelation immediate, remains for extended period.

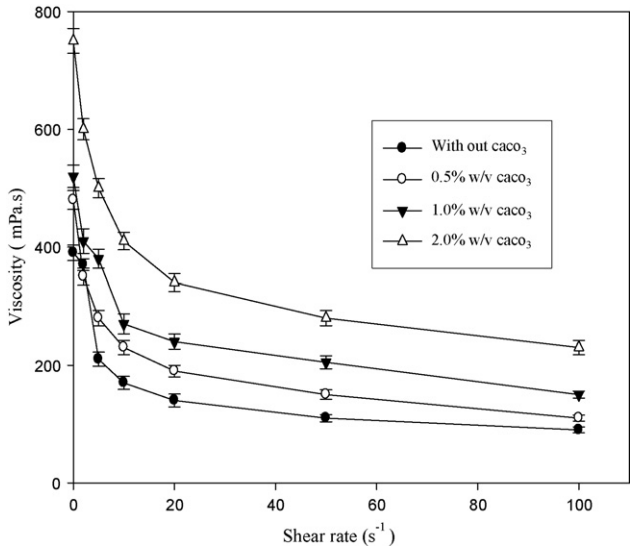

Fig. 2. Effect of calcium carbonate on viscosity of floating in situ gelling systems of amoxicllin at 0.5% (w/v) polymer concentration. Bars represent mean ± S.D. ($n = 3$).

of in situ gelling system containing different concentration of amoxicillin.

The gelation study was conducted in 0.1 N HCl, pH 1.2 (SGF). All the formulations showed instantaneous gelation when contacted with the SGF. The formulation containing calcium carbonate gelled more instaneously than that of 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. It has been shown (Tang et al., 1996) that divalent (Ca^{++}) ions make stronger gellan gels than monovalent (Na^{+})

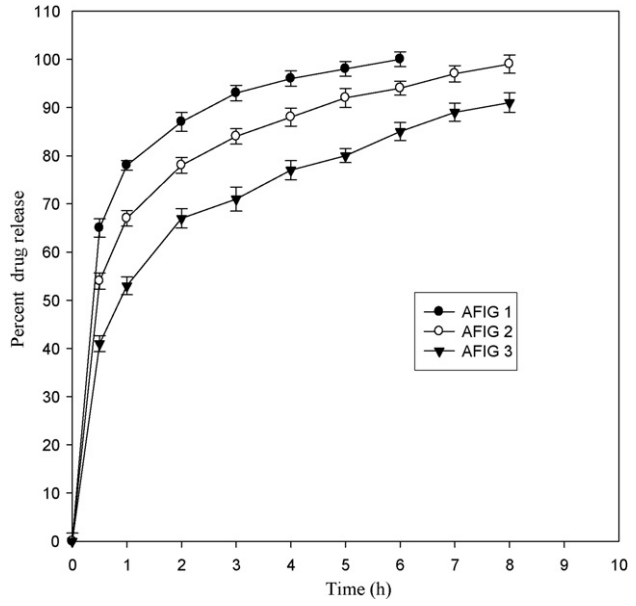

Fig. 3. Effect of polymer concentration on in vitro drug release from amoxicillin floating in situ gelling systema contaning 0.5 % (w/v) calcium carbonate in SGF (pH 1.2). Bars represent mean ± S.D. ($n = 3$).

ions. Johnson and Medlin (1985) investigated the mean maximum breaking strength of rafts formed using a range of alginates, carbonates ($CaCO_3$) and bicarbonates ($NaHCO_3$). In case of alginate, the gel strength of $NaHCO_3$-treated rafts was 44.6 mN, but the gel strength of $CaCO_3$-treated rafts was 58.9 mN.

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 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 vehicle 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.

4.3. Floating properties

The floating ability of the prepared formulations was evaluated in SGF. 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 evaluated and are shown in Table 1. Formulations containing calcium carbonate demonstrated excellent floating ability, while formulations not containing this agent settled at the bottom of the medium.

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 producing buoyant formulation and then calcium ion reacted with gellan 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 (Grasdalen and Smidsroed, 1987; Chandrasekaran et al., 1988; Chandrasekaran and Thailambad, 1990). The floating ability of the formulation mainly depends on calcium carbonate and gellan concentrations. The lowest level of calcium carbonate which produced a buoyant gel system for the duration of drug release study was found to be 0.5% (w/v) at all polymer levels. At 0.25% (w/v) calcium carbonate, the gel was not able to retain the released carbon dioxide in the gel network. Hence, floating was not seen in the formulations containing less than 0.5% (w/v) calcium carbonate 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 CO_2, content at increase 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 and Kim, 2000).

Various drugs loading did not produce any significant change in floating properties.

4.4. In vitro drug release

The effect of polymer concentration on in vitro drug release from in situ gels is shown in Fig. 3. A significant ($P < 0.01$) decrease in the rate and extent of drug release was observed with the increase in polymer concentration in in situ gels and is attributed to increase in the density of the polymer matrix and also an increase in the diffusional path length which the drug molecules have to traverse. The release of drug from these gels was characterized by an initial phase of high release (burst effect). However, as gelation proceeds, 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 (Lemoine et al., 1998). The initial burst effect was considerably reduced with increase in polymer concentration.

In the absence of calcium and 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.

Drug release profiles was compared between control formulation (formulation without calcium carbonate) and formulations containing different concentrations of calcium carbonate. Percentage drug release was always higher for control formulations than calcium carbonate containing formulations (Fig. 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

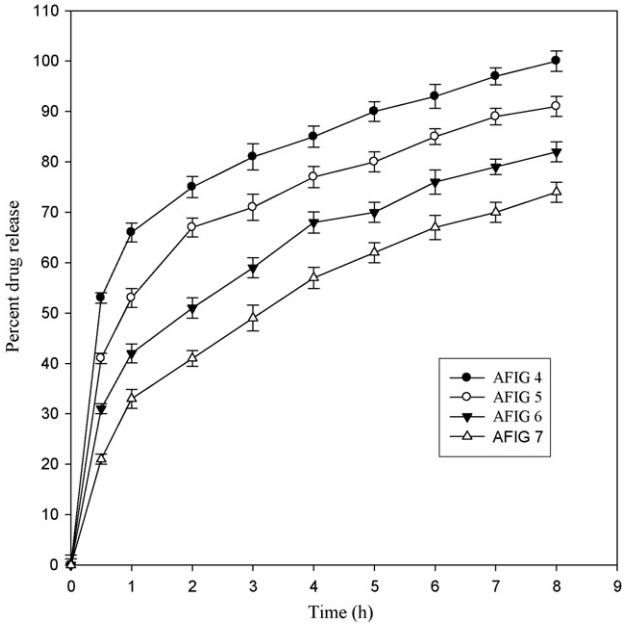

Fig. 4. Effect of calcium carbonate concentration on in vitro drug release from amoxicillin floating in situ gelling system at 1.0 % (w/v) polymer concentration in SGF (pH 1.2). Bars represent mean ± S.D. ($n = 3$).

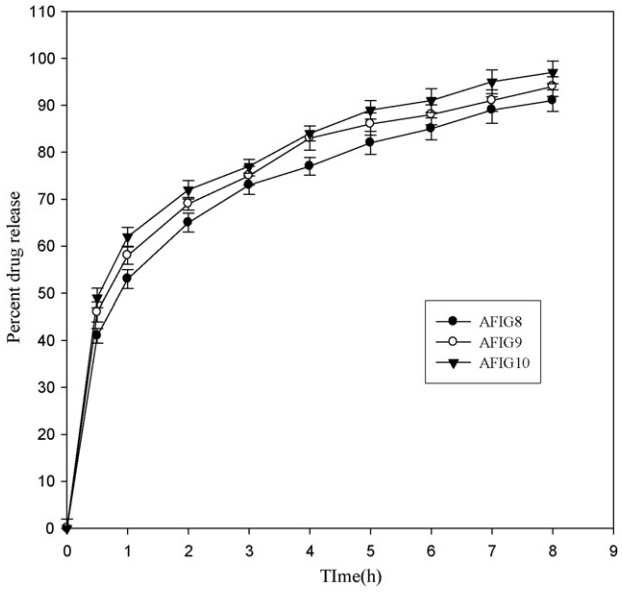

Fig. 5. Effect of drug concentration on in vitro drug release from amoxicillin floating in situ gelling system conatinng 1.0 % (w/v) polymer and 0.5 % (w/v) calcium carbonate in SGF (pH 1.2). Bars represent mean ± S.D. ($n = 3$).

divalent cation Ca^{++}. With increase in calcium carbonate concentration in formulations decreased percentage of drug release was observed (Fig. 4). Effects of drug loading on in vitro drug release from in situ gel formulation are shown in Fig. 5. The results indicated that there was no significant difference ($P > 0.01$) between the rate and extent of amoxicillin release from the floating in situ gels containing different amoxicillin concentration.

The release data from floating in situ gels over the whole time period 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:

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

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. The linear regression analyses are summarized in Table 2. The examination of coefficient of determination (r^2) values for different formulations indicated that drug release followed the diffusion control mechanism from the AFIG.

A more stringent test was used to distinguish between the mechanisms of drug release. Release data were analyzed according to the empirical equation (Higuchi, 1962; Cardinal, 1984) $Q(t) = a \times t^n$ where $Q(t)$ is fraction of drug released after time 't', 'a' a coefficient values and 'n' is release exponent. Values for coefficient of determinations 'r^2' and the release exponent 'n' are listed in Table 2. The values 'n' were in the range of 0.4120–0.6120 which was further indicative of the drug release following a diffusion control mechanism.

Table 2
In vitro drug release kinetic of amoxicillin from floating in situ gelling systems

Batches	Drug release kinetic, correlation coefficients (r^2)			Release exponent (n)
	Zero order	First order	Higuchi	
AFIG1	0.9124	0.9845	0.9951	0.472
AFIG2	0.9025	0.9874	0.9942	0.492
AFIG3	0.9452	0.9773	0.9944	0.535
AFIG4	0.9145	0.9773	0.9939	0.561
AFIG5	0.9012	0.9625	0.9990	0.514
AFIG6	0.9312	0.9803	0.9803	0.485
AFIG7	0.9551	0.9654	0.9954	0.612
AFIG8	0.8941	0.9830	0.9830	0.456
AFIG9	0.8994	0.9879	0.9879	0.412
AFIG10	0.8824	0.9863	0.9931	0.459

Mean ± S.D. ($n = 3$).

4.5. In vivo H. pylori clearance

In vivo evaluation of AFIG was carried out with an animal model—Mongolian gerbils infected with human *H. pylori*. The in vivo clearance data of *H. pylori* after multiple administration of amoxicillin-in situ gel and the amoxicillin suspension under fed conditions (amoxicillin doses at 1, 4, 10 and 40 mg/kg) are presented in Table 3. In the control group receiving no drug, around 10^8 viable bacteria colonized the stomach. The mean bacterial count in gerbil's stomach after oral administration of the amoxicillin suspension decreased as the dose of amoxicillin increased; however, complete clearance of *H. pylori* was not obtained even with the highest dose. This is because of the short residence time of amoxicillin suspension in the stomach and the low concentration of amoxicillin reaching the bacteria under the gastric mucus layer. Amoxicillin has a low MIC for *H. pylori* (Goodwin et al., 1986; Rauws et al., 1988) and low luminal anti-*H. Pylori* activity (Tytgat, 1994). However, compared to other antibacterials (clarithromycin and metronidazole), it takes several hours for amoxicillin to kill *H. pylori* (Flamm et al., 1996). The residence time of amoxicillin in the stomach after oral administration of the conventional dosage form is expected to be short (Cooreman et al., 1993). Therefore, the resulting insufficient duration of contact with the gastric mucosa by conventional dosage form of amoxicillin may be the reason for the incomplete eradication of *H. pylori*. The mean bacterial count after 3 days of treatment with AFIG with an amoxicillin dose of 1.0 mg/kg was almost equal to that of amoxicillin suspension at the dose of 10 mg/kg, which is significantly lower than that of amoxicillin suspension which clearly indicates that due to stomach specific delivery of amoxicillin from in situ gel, it is equally effective at much lower dose than the amoxicillin suspension. On other hand, anti-*H. pylori* therapy with AFIG at amoxicillin dose of 10 and 40 mg/kg, the bacteria were completely cleared in gerbil's stomachs and no bacterial colony was detected in the original dilution from all gastric sample from these groups indicating 100% clearance rate. This is because of the longer residence time of AFIG in the stomach, which enabled high concentration of amoxicillin to reach the bacteria underlying the gastric mucosal layer. The effect of the therapy was further conformed by PCR technique, which has the propensity to detect bacteria more sensitively than microbial culture method. In amoxicillin suspension group and control group, the PCR product was clearly detectable at a concentration of 10^4 dilution of the original DNA extract (Fig. 6a). In contrast PCR sample was barely detectable in the original DNA extract at amoxicillin dose of 1 and 4 mg/kg and was undetectable in the original DNA extract at amoxicillin dose of 10 and 40 mg/kg from the AFIG treated group (Fig. 6b). These results clearly indicate that amoxicillin administered in the form of AFIG more effectively clears *H. pylori* from gerbils stomach than amoxicillin administered in the form of a suspension. Further, the dose of AFIG required to achieve total clearance was 10 times less than that of the amoxicillin suspension.

Table 3
Effect of repetitive administration of amoxicillin suspension and amoxicillin floating in situ gel formulations against gastric infection caused by *H. pylori* in Mongolian gerbils

Formulations	Dose (mg/kg)[a]	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
Amoxicillin suspension			
	1	0/6(0)	7.56 ± 0.52
	4	0/6(0)	5.59 ± 0.85
	10	2/6(33)	4.08 ± 0.78*
	40	4/6 (66)	2.02 ± 0.51**
Amoxicillin floating in situ gel formulations			
AFIG3	1	2/6(33)	5.16 ± 0.27*
AFIG8	4	5/6(77)	3.41 ± 0.98**
AFIG9	10	6/6(100)	ND
AFIG10	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 days as amoxicillin.
[b] Bacterial cell counts less than $10^{1.45}$ CFU were considered to be $10^{1.45}$ to calculate the mean. Values are means ± S.E.
* $P < 0.05$.
** $P < 0.01$ (both significant level in reference to control).

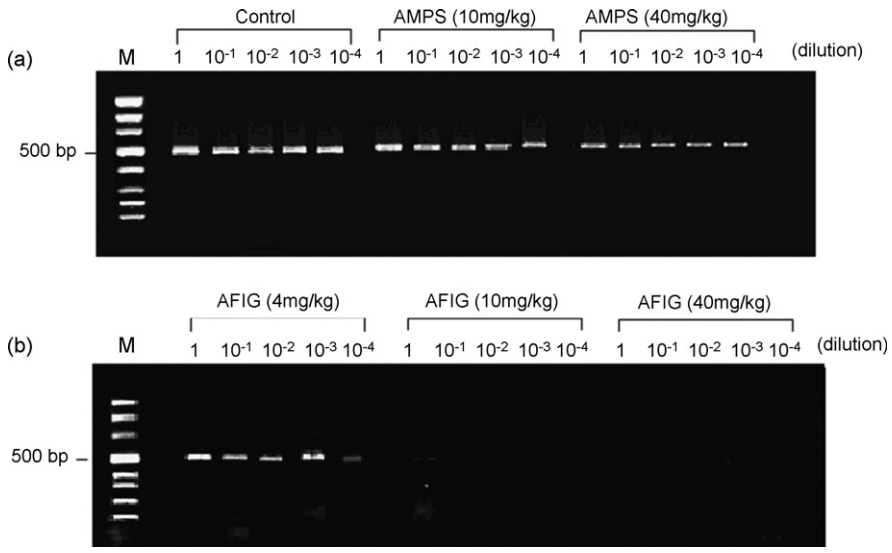

Fig. 6. Polymerase chain reaction (PCR) amplification of the 16S rRNA gene of *H. pylori* infected in gerbil stomachs. DNA from the homogenates of each stomach was serially diluted 10-fold (1–10^{-4} dilution) and 1 μl of each sample was amplified with the 16S rRNA gene-specific primers. (a) PCR detection *H. pylori* from amoxicillin suspension (AMPS) treated gerbils stomach and (b) PCR detection *H. pylori* from amoxicillin floating in situ gel (AFIG) treated gerbils stomach. M, molecular marker.

5. Conclusion

In conclusion, AFIG has feasibility of forming gels in stomach and sustaining the drug release from the gels over the period of at least 8 h. Further, the prepared in situ gels were effective in clearing *H. pylori* in infected gerbils stomach at a dose level, which was 10-fold less than the amoxicillin suspension, which is important from the viewpoint of reducing adverse effect during the therapy.

With regard to the single multiple therapy, the experimental data obtained in the present animal model are in good agreement with clinical data (Hirayama et al., 1996) demonstrating that the infected gerbil can sufficiently mimic the pathogenic feature of human infected with *H. pylori*. However, some problems remain to be solved. For example, the doses of amoxicillin used in present study differ greatly from those used in clinical trials (Labenz et al., 1994). Amoxicillin was administered to the animals at lower doses compared with clinical dose, since it has been found that this drug is toxic to gerbil at doses exceeding 1 mg/kg (Logan et al., 1994). Another problem is that we determined the bacterial clearance only 3 days after the therapy in this study. This may not be sufficient, and schedules for the assessment of bacterial clearance and eradication need to be optimized for each experimental model, which will form the basis for our future work. In spite of the deficiencies in the study design, the results indicate that AFIG could potentially be viewed as a site-specific delivery system of amoxicillin for the treatment of peptic ulcer disease caused by *H. pylori*.

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