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Original research paper

Preparation and *in vitro* characterization of gellan based floating beads of acetohydroxamic acid for eradication of *H. pylori*

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Gellan based floating beads of acetohydroxamic acid (AHA) were prepared by the ionotropic gellation method to achieve controlled and sustained drug release for treatment of *Helicobacter pylori* infection. The prepared beads were evaluated for diameter, surface morphology and encapsulation efficiency. Formulation parameters like concentrations of gellan, chitosan, calcium carbonate and the drug influenced the *in vitro* drug release characteristics of beads. Drug and polymer interaction studies were carried out using differential scanning calorimetry. Chitosan coating increased encapsulation efficiency of the beads and reduced the initial burst release of the drug from the beads. Kinetic treatment of the drug release data revealed a matrix diffusion mechanism. Prepared floating beads showed good antimicrobial activity (*in vitro H. pylori* culture) as potent urease inhibitors. In conclusion, an oral dosage form of floating gellan beads containing AHA may form a useful stomach site specific drug delivery system for the treatment of *H. pylori* infection.

Keywords: acetohydroxamic acid, gellan beads, controlled release, floating drug delivery, stomach-specific delivery, *Helicobacter pylori*

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Drug delivery systems that can precisely control the release rate of target drug to a specific body site have had an enormous impact on the healthcare system. In addition to drug formulations that deliver the drug for a prolonged period of time, it is important for efficient therapy to achieve spatial placement of the dosage form in the gastrointestinal tract (GIT). Site-specific drug delivery, using novel formulation designs, would improve local therapy in the GIT, optimize systemic absorption and minimize premature drug degradation (1). Stomach-specific antibiotic drug delivery, for instance, would be highly beneficial in the treatment of *Helicobacter pylori* infection in peptic ulcer disease (2–4).

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In recent years, the number of individuals (more than 60% of adults in industrialized countries) suffering from acid peptic disease and gastric adenocarcinoma caused by *H. pylori* has increased (5). Current strategies aimed at eradication of *H. pylori* infection from patients rely on triple therapy that includes an anti-secretory agent in combination with two antibiotics, but these regimes are not wholly effective and patient compliance, side effects and bacterial resistance are major drawbacks of the therapy. This is due to the fact that the organism remains exclusively on the luminal surface of the gastric mucosa under the mucous gel layer. Thus, the access of antimicrobial drugs to the site is restricted both from the stomach and from the gastric blood supply (6, 7). Also, the antibiotics are not delivered to the site of infection in effective concentration and in fully active form by the conventional drug delivery systems (4). Despite the multi-antibiotic therapy, different therapeutic strategies have been examined to completely eradicate *H. pylori* from the stomach. Drug delivery to the site of residence in the gastric mucosa may improve the efficacy of the current and emerging treatments. Gastric retentive delivery systems potentially allow increased penetration of the mucus layer and therefore increase drug concentration at the site of action (6, 8).

To overcome the problems, we have proposed a concept based on a floating drug delivery system with floating site-specific drug delivery. It is necessary to design a drug delivery system that could not only curtail and alleviate the shortcomings of conventional drug delivery vehicles, but also deliver the antimicrobial agent to the infected cell lines. The drug, acetohydroxamic acid (AHA) inhibits cytoplasmic, which plays an important role in the chemotactic motility of *H. pylori* (9). As AHA is a small molecule (molecular mass, 75.07), it can permeate intact bacterial cells and effectively inhibit the urease activity of *H. pylori.* Freely diffusible AHA inhibits over 95% of urease activity after 10 min (10, 11). Gellan gum is a bacterial anionic deacetylated polysaccharide secreted by *Pseudomonas elodea*. Due to its characteristic property of temperature-dependent and cation-induced gelation (12), gellan was selected as a matrix polymer for the preparation of AHA beads in the formulation. The present work is therefore aimed developing of anti-*H. pylori* agent (antiurease) AHA-loaded gellan floating beads coated with chitosan for stomach-specific drug delivery for eradication of *H. pylori*.

EXPERIMENTAL

Materials

Acetohydroxamic acid (AHA) and gellan (Gelrite) were purchased from Sigma-Aldrich Chemicals (India). Chitosan was a generous gift sample of the Centre for Fisheries and Research Institute (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

Preparation of beads. – The beads were prepared by the ionotropic gelation technique. Gellan solution (0.25–1.0%, *m/V*) was prepared by dissolving the gellan in deionized wa-

ter by heating at 90 °C. Different concentrations of the drug (0.1–2.0%, *m/V*) and calcium carbonate (0.5–2.0%, *m/V*) were dissolved/dispersed uniformly in 50 mL of gellan solution below 40 °C *m/V* under 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 syringe needle into 100 mL of calcium chloride solution (1.5%), which was kept under stirring to improve the mechanical strength of the beads and also to prevent aggregation of the formed beads. Immediate formation of small gelled beads took place; after 5 minutes of curing time, the formed beads were collected by filtration and dried at 40 °C. The gellan beads coated with chitosan were prepared by dropping the gellan slurry containing different amounts of drug and calcium carbonate into 100 mL of calcium chloride (1.5%, *m/V*) with chitosan dissolved in it at various concentrations (0.2–0.6%, *m/V*). Then the beads were collected and dried as mentioned above.

Morphology and particle size analysis. – Particle size of the prepared beads was 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 the mean diameter of beads. The shape and surface morphological examination of the surface structure of dried beads were carried out by scanning electron microscopy (SEM-JEOL Model 8404, Japan at magnification of 500x).

In vitro *floating properties of gellan beads.* – The *in vitro* floating study was performed using a USP 24 dissolution apparatus II (13) having 500 mL of simulated gastric fluid (SGF, pH 1.2). 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 the medium was agitated with a paddle at 50 rpm. After agitation, the beads that floated on the surface of the medium and those that settled down at the bottom of the flask were recovered separately. The floating percentage was estimated by visual observation.

Entrapment efficiency of beads. – The drug content in gellan beads was determined by the reported method with a slight modification (14). Briefly, the dried beads (100 mg) were allowed to disintegrate in 50 mL of phosphate buffer (pH 7.2) for 4 h. The whole dispersion of beads was sonicated at 125 W for 30 min (Imeco Sonifier, Imeco Ultrasonics, India) and the solution was filtered through a 0.45-μm filter. Then the polymeric debris was washed twice with fresh solvent (phosphate buffer) to extract any adhering drug. The drug content of filtrate and washings was determined spectrophotometrically at 503 nm (Shimadzu, UV/Visible 1601, Japan). Each sample determination was made in triplicate.

Differential scanning calorimetry (DSC). – 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, USA). About 3.0 mg of sample was placed in an aluminium pan and then hermetically sealed with an aluminium lid. The thermograms were obtained at a scanning rate of 5 °C min^{-1} over a temperature range of 40 to 150 °C under an inert atmosphere flushed with nitrogen at a rate of 20 mL min^{-1}. All tests were performed twice.

Measurement of in vitro drug *release.* – The release of AHA from the floating beads was determined using a USP 24 dissolution test apparatus I with a basket (13). A weighed quantity of beads equivalent to 100 mg of AHA was placed in the dissolution basket and the basket was placed in 500 mL dissolution medium. The dissolution medium having 500 mL of simulated gastric fluid (pH 1.2) was maintained at 37 ± 0.5 °C (3). Five mL of sample was withdrawn at different time intervals and replaced with the same volume of freshly prepared dissolution medium. The drug content was measured at 503 nm. These experiments were conducted in triplicate.

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 FBA_2 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/held 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 and 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).

In vitro *growth inhibition studies.* – The bacterial strain used in this study was originally isolated from a hospitalized human patient (aged 50 years) with gastric ulcer. *In vitro* growth inhibition studies were performed on developed system using a broth culture of *H. pylori*. *H. pylori* broth culture was preformed in a 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* strain was grown in brucella broth at 37 °C after 7 days in a microaerophilic atmosphere (5% O_2, 10% CO_2, 85% N_2). Growth of the bacteria was monitored by measuring the optical density of broth cultures spectrophotometrically at 600 nm. The number of bacteria was determined by OD with one optimal density unit corresponding to 10^8 colony-forming unit (CFU) mL^{-1}. The colonies were identified as *H pylori* by morphology and urease activity (2). To study the effect of formulations on *H. pylori* growth inhibition (GI), 10 mL of nutrient broth was inoculated with a loopful of the *H. pylori* from stock culture to make a final culture of 10^8 CFU mL^{-1}. AHA plain drug and different formulations were added to the tubes and all the tubes were incubated at 37 °C in a microaerophilic atmosphere. Acetohydroxamic acid was

used at a final concentration of 14 mmol L^{-1} (14 mmol L^{-1} is approximately four fold of the reported MIC_{50} for *H. pylori* urease) (10).

The culture containing tubes were shaken at 100 rpm at 37 °C in a microaerobic atmosphere in an incubator. Then, 100 µL of nutrient broth containing AHA and different AHA formulations was removed at various time points (4, 8, 12 and 24 h) 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, USA). The viable cell counts for each sample were calculated by counting the number of colonies on the agar plates.

Statistical analysis

Statistical evaluation of the data was performed using the analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Particle size and morphology of beads

The formulation composition and physico-chemical properties of the various batches of the prepared AHA floating beads are shown in Tables I and II, respectively. The scanning electron micrographs (SEM) of the beads are shown in Figs. 1a and b. The SEM

Table I. Formulation variables of the prepared floating beads of acetohydroxamic acid

Batch	Gellan concentration (%, *m/V*)	Chitosan concentration (%, *m/V*)	Calcium carbonate concentration (%, *m/V*)	AHA (%, *m/V*)
FBA_1	0.25	–	1.00	0.50
FBA_2	0.50	–	1.00	0.50
FBA_3	1.00	–	1.00	0.50
FBA_4	0.25	0.40	1.00	0.50
FBA_5	0.50	0.40	1.00	0.50
FBA_6	1.00	0.40	1.00	0.50
FBA_7	0.50	0.20	1.00	0.50
FBA_8	0.50	0.60	1.00	0.50
FBA_9	0.50	0.40	0.00	0.50
FBA_{10}	0.50	0.40	0.50	0.50
FBA_{11}	0.50	0.40	2.00	0.50
FBA_{12}	0.50	0.40	1.00	0.10
FBA_{13}	0.50	0.40	1.00	1.00
FBA_{14}	0.50	0.40	1.00	2.00

Table II. Physico-chemical characteristics of the prepared floating beads of acetohydroxamic acid

Batch	Diameter (mm)[a,b]	Entrapment efficiency (%)[a,c]	Floating lag time (min)[a,c]	Floating ability (%)[a,c]	Drug content (mg)[a,c,d]
FBA_1	0.72 ± 0.04	44.30 ± 2.49	6.51 ± 0.61	88.25 ± 2.42	42.47 ± 1.98
FBA_2	0.79 ± 0.04	48.81 ± 3.98	5.26 ± 1.02	86.41 ± 2.14	45.21 ± 3.14
FBA_3	0.86 ± 0.02	56.21 ± 2.52	4.67 ± 0.24	88.24 ± 1.82	57.42 ± 2.14
FBA_4	0.78 ± 0.06	56.25 ± 2.65	5.09 ± 1.03	86.26 ± 3.12	55.30 ± 2.47
FBA_5	0.85 ± 0.04	65.78 ± 3.51	5.79 ± 1.13	87.23 ± 2.13	67.20 ± 2.62
FBA_6	0.89 ± 0.04	73.71 ± 2.80	6.15 ± 1.37	85.82 ± 1.98	74.17 ± 2.43
FBA_7	0.74 ± 0.05	61.80 ± 4.12	6.28 ± 0.23	87.49 ± 2.19	58.67 ± 2.98
FBA_8	0.88 ± 0.03	58.72 ± 3.56	6.45 ± 1.02	92.36 ± 2.54	57.35 ± 2.61
FBA_9	0.85 ± 0.04	59.83 ± 1.65	none	none	57.81 ± 1.94
FBA_{10}	0.86 ± 0.05	58.78 ± 4.03	10.41 ± 0.32	71.51 ± 2.78	59.34 ± 2.68
FBA_{11}	0.91 ± 0.02	60.23 ± 2.82	4.89 ± 0.36	100	61.62 ± 2.44
FBA_{12}	0.80 ± 1.01	57.35 ± 2.41	6.12 ± 1.02	86.56 ± 2.63	55.40 ± 2.18
FBA_{13}	0.84 ± 1.01	60.23 ± 2.82	6.07 ± 0.42	87.72 ± 2.12	61.67 ± 2.35
FBA_{14}	0.85 ± 0.10	52.35 ± 2.41	5.61 ± 0.39	87.52 ± 2.79	51.32 ± 2.41

[a] Mean ± SD.
[b] $n = 20$.
[c] $n = 3$.
[d] Drug content of each 100 mg of beads.

results revealed that all the AHA-loaded floating beads were discrete and spherical in shape with rough outer surface (Figs. 1a and b). However, the chitosan coated AHA beads had outer rough surface with a number of minor wrinkles (Fig. 1b). This is due to the formation of a thin layer coat of chitosan over the beads, which caused several wrinkles during the drying due to dehydration of the chitosan membrane surrounding the beads (Fig. 1b). The bead diameter varied from 0.72 ± 0.04 to 0.91 ± 0.02 mm for different batches. The results indicate that as the amount of gellan and calcium carbonate increased, the size of beads also proportionally increased (Table II). This could be attributed to the increase in micro-viscosity of the polymeric dispersion due to increasing gellan concentration, which eventually led to formation of bigger beads. The mean diameter of prepared beads marginally increased with an increase in drug loading and chitosan concentration. This could be attributed to drug solubility in water and formation of a thin chitosan coating over the beads due to its ionic interaction, respectively.

In vitro *floating properties*

The floating ability of the prepared formulations was evaluated in SGF (pH 1.2) using a USP 24 dissolution apparatus II (13). The time the formulation took to emerge on the medium surface (floating lag time) and the percentage of floating beads on the dissolution medium surface were evaluated and are shown in Table II. Upon contact with an acidic medium, gelation and cross-linking by Ca^{2+} ions occurred to provide a gel barrier

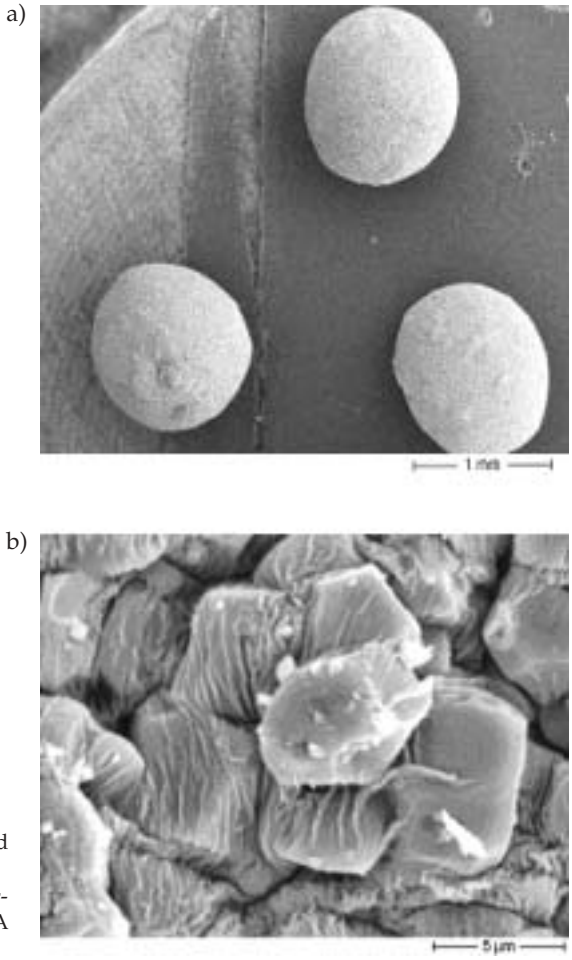

Fig. 1. SEM photograph of the prepared floating beads of acetohydroxamic acid: a) prepared AHA beads (FBA_2), b) surface morphology of chitosan coated AHA beads (FBA_2).

at the surface of the formulation. The calcium carbonate effervesced, releasing carbon dioxide and calcium ions. The released carbon dioxide was entrapped in the gel network producing buoyant formulation and then calcium ion reacted with gellan producing a cross-linked three-dimensional gel network that restricted further diffusion of carbon dioxide and drug molecules and resulted in an extended period of floating and drug release (16–18). The floating ability of the formulation mainly depended on calcium carbonate and gellan concentrations. The control beads (without calcium carbonate) sank uniformly in the medium. The beads containing 0.5 to 2.0% of the gas-forming agent demonstrated good floating ability (71–100% of floating). The floating lag time for this system was in the range of 4–10 min. The lowest concentration of calcium carbonate which makes the beads float throughout the drug release study was found to be 0.5% (*m/V*) at all polymer levels.

On increasing the calcium carbonate concentration, the floating lag time was reduced and the duration of floating was increased (Table II). The increase in the amount of Ca^{2+} and consequently in the amount of CO_2 evolved are responsible for the observed reduction in the floating lag time and increased duration of floating. Similarly, an increase in the polymer concentration resulted in decreased lag time of the prepared beads (Table II). In SGF, the beads containing 2.0% (*m/V*) calcium carbonate exhibited a good floating ability; about 100% beads floated after the lag time of approximately 4 min (Table II). On increasing the $CaCO_3$ concentration from 0.75 to 2.5% (*m/V*), the floating lag time of AHA beads was reduced from 10 to 4 min (Table II). Almost all the prepared beads were floating for > 8 h in SGF pH 1.2 (data not shown). Different drug concentration did not produce any significant effect on floating properties of the beads; the beads remained buoyant even after the buoyancy test period.

Entrapment efficiency

The effects of various formulation parameters on the entrapment efficiency of the prepared floating beads are shown in Table II. The entrapment efficiency of the prepared floating beads varied from 44.3% for batch FBA_1 (gellan 0.25%, *m/V*, no chitosan coat) to 73.7% for batch FBA_6 (gellan 1.0%, *m/V* coated with chitosan 0.4%, *m/V*). The entrapment efficiency increased significantly ($p < 0.05$) with increasing polymer concentration, as shown in Table II. This is because the increase in the gellan concentration resulted in the formation of larger beads entrapping more drug. The entrapment efficiency of beads increased with increasing the drug loading up to 1.0% (*m/V*). However, increasing AHA concentration above 2.0% (*m/V*) caused a marginal decrease in the incorporation efficiency, suggesting that the quantity of gellan becomes insufficient to entrap the drug. The entrapment efficiency was also found to be proportionally increased with increasing chitosan concentration. This is due to the increasing thickness of chitosan coat formed over the beads which may encapsulate a larger amount of drug. This result is well correlated with similar results reported earlier for alginate-chitosan beads of timolol maleate (19). The method adopted for the preparation of beads could be responsible for the observed higher entrapment efficiency. No significant effect on entrapment efficiency of beads was observed with increasing concentration of calcium carbonate (Table II).

DSC studies

In an effort to investigate the possible physical and chemical interactions between drug and polymer, we have analyzed: (*i*) pure acetohydroxamic acid, (*ii*) placebo beads and (*iii*) drug-loaded beads using modulated DSC. The results are displayed in Fig. 2. The DSC thermogram showed a sharp endothermic peak at 89.22 °C for pure AHA as the melting point of the drug (Fig. 2a). In placebo beads, thermal transition at 246.60 °C can be seen, which is attributed to the melting point of the gellan polymer. In the DSC thermogram of the drug-loaded beads, the endothermic peak was observed at 88.32 °C as the melting point of the drug (Fig. 2c). The evaluation of the thermograms clearly revealed no physical interaction between the polymer and the drug in the beads. The analysis of thermograms revealed no physical interaction between the polymer and the drug in the prepared beads.

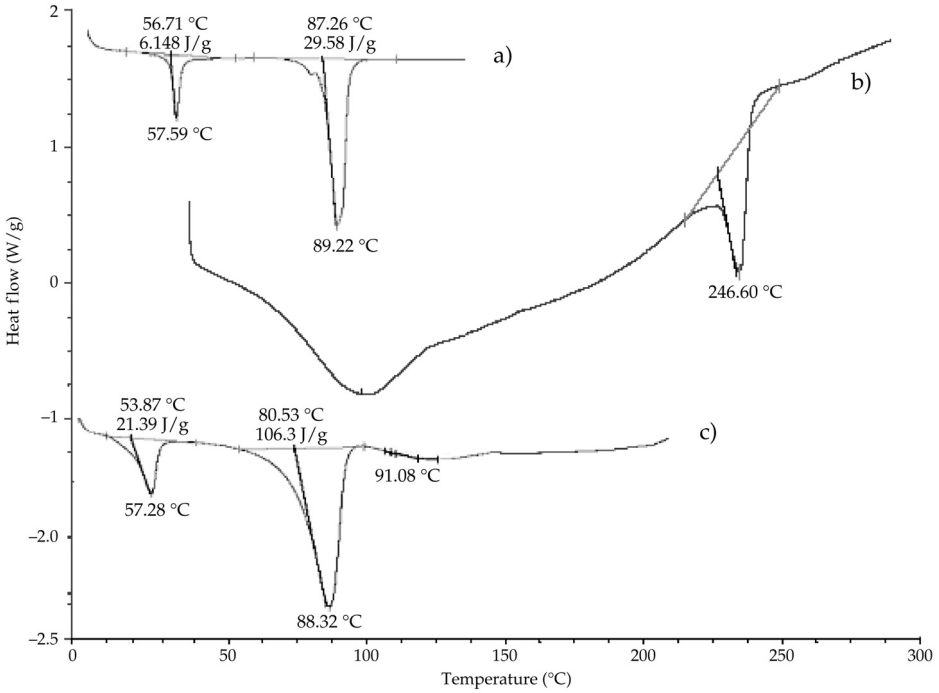

Fig. 2. DSC theromograms of: a) pure acetohydroxamic acid, b) placebo beads, c) acetohydroxamic acid-loaded beads (FBA_2).

In vitro *drug release*

The *in vitro* drug release profiles of gellan floating beads of AHA with different polymer concentrations are shown in Figs. 3a and b. The rate and extent of AHA release from floating beads significantly decreased ($p < 0.05$) with an increase in gellan concentration. This could be attributed to the increase of gellan matrix density and in the diffusion path length which the drug molecules have to traverse (by formation of bigger sized beads). The drug release from these beads was characterized by an initial phase of high release (burst effect) due to good solubility of AHA in water. However, as gelation proceeded (cross-linking of gellan with Ca^{2+} ions from calcium carbonate), the remaining drug was released at a slower rate followed by a phase of moderate release. This bi-phasic pattern of release is a characteristic feature of matrix diffusion kinetics (20). The initial burst effect was considerably reduced with the increase in gellan concentration (Fig. 3a). The initial burst effect from batches of chitosan-coated beads (FBA_4, FBA_5 and FBA_6) was considerably reduced when compared to the corresponding batches of chitosan non-coated beads (FBA_1, FBA_2 and FBA_3), as shown in Fig. 3b. The fact is that chitosan coating over the beads resulted in better incorporation efficiency and formed a thick coating layer around the beads. This could be the reason for the observed decrease in the burst effect.

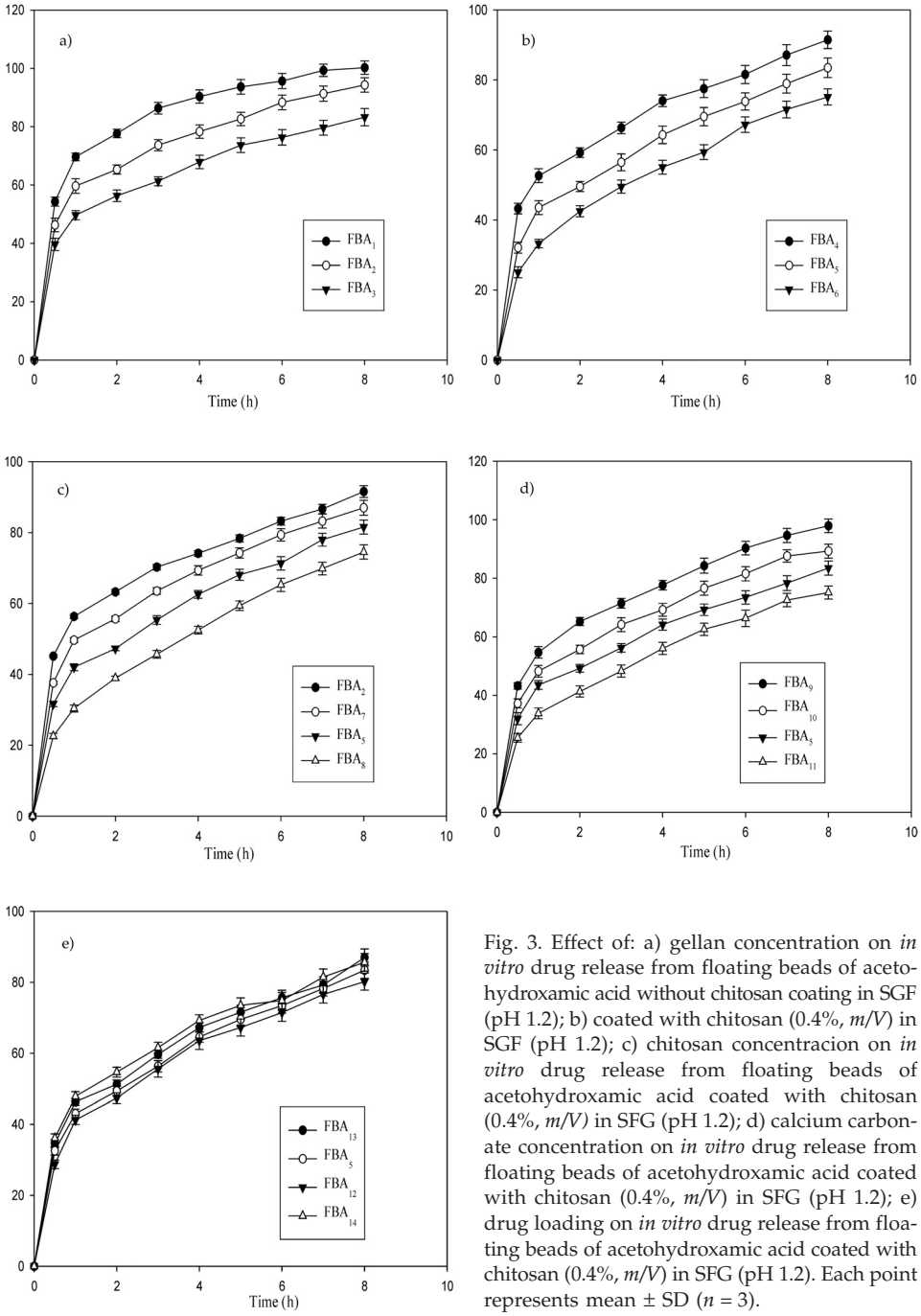

Fig. 3. Effect of: a) gellan concentration on *in vitro* drug release from floating beads of acetohydroxamic acid without chitosan coating in SGF (pH 1.2); b) coated with chitosan (0.4%, *m/V*) in SGF (pH 1.2); c) chitosan concentracion on *in vitro* drug release from floating beads of acetohydroxamic acid coated with chitosan (0.4%, *m/V)* in SFG (pH 1.2); d) calcium carbonate concentration on *in vitro* drug release from floating beads of acetohydroxamic acid coated with chitosan (0.4%, *m/V*) in SFG (pH 1.2); e) drug loading on *in vitro* drug release from floating beads of acetohydroxamic acid coated with chitosan (0.4%, *m/V*) in SFG (pH 1.2). Each point represents mean ± SD ($n = 3$).

Fig. 3c indicates the effect of chitosan concentration on the release properties of AHA from gellan beads. An increase in chitosan concentration in beads caused a significant ($p < 0.05$) retardation in the drug release of the beads as a result of an increase in the thickness of the coat of chitosan over the beads, thereby increasing the distance traveled by the drug molecule through the chitosan coat. Release rates of AHA from gellan beads with different amounts of calcium carbonate are shown in Fig. 3d. An increase in calcium carbonate content prolonged the release of AHA from the gellan matrix. This effect may be due to the internal ionotropic gelation effect of calcium carbonate. In acidic medium, the calcium carbonate dissolves and the ionized Ca^{2+} ions then promote internal gelation by cross-linking with the gellan and retarding the drug release from gellan matrix (21).

The effect of drug loading on AHA release from the prepared beads is shown in Fig. 3e. The results indicate that different drug loadings of beads did not produce any significant difference in the rate and extent of drug release from beads. However, an initial high release was observed with the formulation batch FBA_{14} with higher drug loading.

In order to investigate the mechanism of drug release, the data were fitted to models representing zero-order, first order and Higuchi's square root of time (22). The examination of the coefficient of determination (R^2) indicated that drug release from the prepared beads followed a diffusion controlled mechanism, since the R^2 values for Higuchi's square root of time (from 0.9661 to 0.997) was always higher compared to zero-order (from 0.8604 to 0.9238) and to the first-order ones (from 0.9126 to 0.9613). 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 the Peppas exponential model (23) $M_t/M_\infty = Kt^n$, where M_t/M_∞ is the fraction of drug released after time t, K is the kinetic constant and n is the release exponent which characterizes the drug transport mechanism. The n values were in the range of 0.3248–0.5653, indicating that all the prepared formulations followed the Fickian diffusion controlled mechanism of drug release.

In vivo *floating efficiency*

The results of X-ray photographs of floating beads at different time intervals in rabbits stomach are shown in Figs. 4a-c. One hour after dosing, the beads showed good floatability (~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. 4b and c). The results clearly indicate that the prepared floating beads of AHA remained buoyant for at least 6 h in rabbits stomach and that they had good floatability *in vivo*.

In vitro *growth inhibition studies*

The effect of different drug-loaded, drug-free formulations (placebo) and plain AHA on bacterial growth was investigated at various time intervals for up to 24 hours and the results are shown in Fig. 5. The antimicrobial effect of formulations and plain drug were determined in terms of percentage growth inhibition (GI), which was calculated as the ratio of optical density (OD) of a given mixture against that of tubes containing *H. pylori*

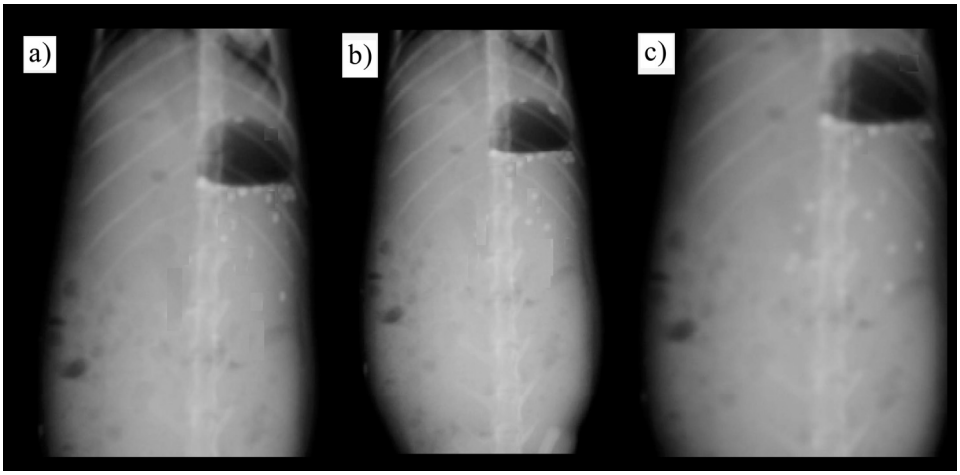

Fig. 4. X-ray photographs of floating beads of AHA in the gastric region of rabbit after dosing of formulations in the fasted state: a) 1 h after dosing, b) 4 h after dosing, c) 6 h after dosing.

alone. Placebo beads (control batch) did not exhibit any significant growth inhibition (Fig. 5).

In order to evaluate the *in vitro* growth inhibition of formulations against *H. pylori*, two batches containing different drug concentrations (FBA_1 and FBA_{12}) have been selected. The growth inhibition of drug loaded formulations was compared with that of the drug alone. The percentage GI values for placebo, AHA, FBA_1 and FBA_{12} were 3.6 ± 1.2, 69.0 ± 5.6, 56.3 ± 5.6 and 49.9 ± 6.7, respectively after 4 h incubation. After 8 hours of incubation with formulations FBA_1 and FBA_{12}, the bacterial growth was reduced to 68.1 ± 6.3% and 60.2 ± 5.2%, respectively (Fig. 5), whereas the AHA pure drug inhibited completely the *H. pylori* growth. The AHA formulations achieved complete growth inhibition only after 12 h of incubation. Continued incubation of *H. pylori* for up to 24 hours in

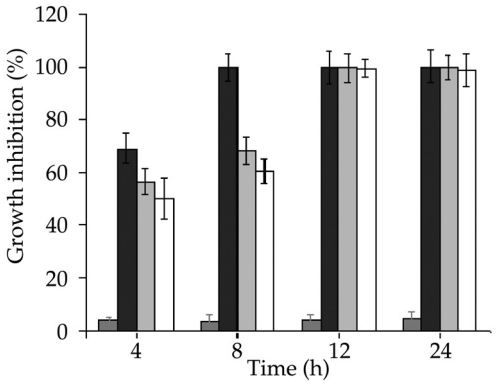

Fig. 5. Percentage of *H. pylori* growth inhibition (mean ± SD, $n = 3$). ■ Placebo beads, ■ AHA plain drug, ■ FBA_{11}, □ FBA_{12}.

the presence of AHA formulations inhibited completely the bacterial growth. This is due to the controlled delivery of AHA from formulations, meaning that the microorganism was exposed to a lower drug concentrations.

The floating beads containing AHA may show more efficacy in *in vivo H. pylori* clearance than pure AHA due to longer residence time of the formulation at the site in stomach where *H. pylori* resided. The results clearly indicate that the formulations showed good inhibition in *in vitro* culture. However, the time required for complete inhibition was shorter for AHA than for AHA formulations because of the direct exposure of *H. pylori* to AHA. From the interaction and adsorption of the AHA formulations, the formulations effectively targeted the drug on the *H. pylori* surface. Particularly, AHA inhibits its cytoplasmic urease that plays an important role in the chemostatic motility of *H. pylori*. Thus, it can be expected that the floating formulations with selected drug (AHA) will abolish all the mechanisms of *H. pylori* survival *in vivo* and may provide better treatment efficacy for *H. pylori* eradication.

CONCLUSIONS

The prepared gellan beads of AHA floated in SGF for a prolonged period of time and sustained drug release from the beads over a period of at least 8 h. The *in vivo* floating efficiency of beads was satisfactory; beads were retained in rabbits stomach for extended periods. The *in vitro H. pylori* inhibition study showed good antimicrobial activity of formulations in *H. pylori* culture.

From the results one can conclude that the gellan based floating beads containing AHA have a promising potential for delivering AHA at the stomach site and may be very useful for *H. pylori* eradication.

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S A Ž E T A K

Priprava i *in vitro* karakterizacija plutajućih zrnaca acetohidroksamske kiseline za iskorjenjivanje *H. pylori*

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Metodom ionotropskog želiranja pripravljena su plutajuća zrnca acetohidroksamske kiseline (AHA) na bazi gelana za kontrolirano i usporeno oslobađanje ljekovite tvari, namijenjena za liječenje infekcija uzrokovanih *Helicobacter pylori*. Pripravljenim zrncima proučavani su dijametar, površinska morfologija i sposobnost inkapsuliranja. Koncentracija gelana, kitozana, kalcijeva karbonata i ljekovite tvari utjecala je na oslobađanje *in vitro*. Interakcija između ljekovite tvari i polimera praćena je diferencijalnom pretražnom kalorimetrijom. Oblaganje zrnaca kitozanom povećalo je učinkovitost inkapsuliranja i smanjilo početno naglo oslobađanje. Oslobađanje ljekovite tvari slijedilo je mehanizam difuzije matriksa. Plutajuća zrnca s AHA pokazala su antimikrobno djelovanje *in vitro* na kulturi *H. pylori* kao snažni inhibitori ureaze. Može se zaključiti da su plutajuća zrnca s AHA na bazi gelana pogodna za specifičnu isporuku u želucu te korisna u terapiji infekcija uzrokovanih *H. pylori.*

Ključne riječi: acetohidroksamska kiselina, zrnca s gelanom, kontrolirano oslobađanje, plutajući pripravci za isporuku lijekova, specifična isporuka u želucu, *Helicobacter pylori*

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