International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.3, No.3, pp 1359-1368, July-Sept 2011

Evaluation of pH trigger oil entrapped buoyant beads of acetohydroxamic acid for eradication of *Helicobacter pylori*

Girish Kumar Tripathi*[1], Satyawan Singh[1], Gopal Nath[2]

[1]Novel Drug Delivery Laboratory, Department of Pharmacy, Saroj Institute of Technology & Management Lucknow India.

[2]Department of Microbiology & Infectious disease, Institute of Medical Science, Banaras Hindu University Varanashi, India.

*Corres.Author: orgpharm@hotmail.com
Phone Number:+91 7376880959

Abstract: Gastroretentive systems can remain in the gastric region for several hours and hence significantly prolong the gastric residence time of drugs. The objective of present investigation is to develop pH sensitive extended and controlled release formulation of acetohydroxamic acid loaded oil entrapped calcium pectinate micro gel beads. Pectin based oil entrapped micro gel beads were prepared by ionic gelation technique. The drug was formulated into micro gel bead with entrapping the castor oil or mineral oil . Gel beads were prepared instantly after dropping of the formulation mixture into calcium chloride solutions. The beads were optimized by coating with ethylcellulose solution. The prepared beads were evaluated with diameter, floating lag time and % encapsulation efficiency , % drug content and drug release. The prepared beads were spherical and were found high drug content and encapsulation efficiency. The formulation exhibited sustained release behaviour and was best fitted in the Peppas model with $n < 0.45$. Subsequent coating of the selected batch of microbeads exhibited zero-order sustained pattern of the drug release up to 8 hr. The goodness of fitting the release data to the model was indicated by high correlation coefficient value and small errors. The optimized batch was found to be higher gastric retention efficiency in the albino rats and eradication efficiency for isolated *Helicobacter pylori* (*H. pylori*) strain . The results indicate that the prepared optimize bead was promising for the maintenance of effective concentration of the drug in the vicinity of *H. pylori* infection and was effective in term of complete termination of the infection from the stomach..
Keywords: Acetohydroxamic acid, *Helicobacter pylori*, Residential time, pH Sensitive delivery, Ethyl cellulose.

INTRODUCTION

Oral route drawn special attentions for the delivery of anti infective agents that are needed to produce local action in the gastrointestinal tract. This route of administration of drug usually highly compliance by patients due to easy in administration. Resent scientific reports reveals that increased interest in novel dosage form for the targeting of different part of the gastrointestinal tract which could be retain at the site of action for predetermined time. This approach especially most attracted for the delivery of anti-infective agent for the targeting of local microbial cell lines of the gastrointestinal tract.

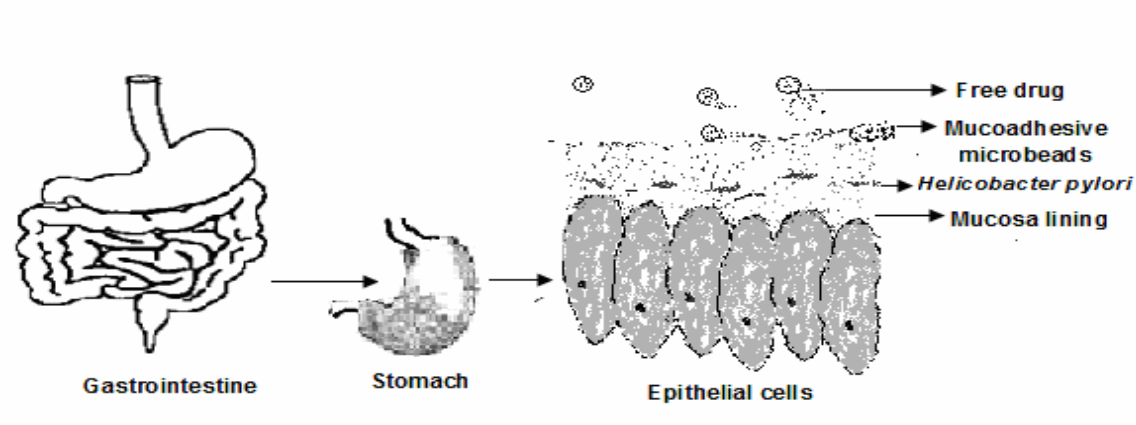


Figure 1. Schematics of targeted drug delivery approach for *H. pylori* eradication located within the stomach.

A problem frequently encountered with conventional oral dosage forms is the inability to localise release of drug in the stomach and proximal portion of the small intestine[1,2] .The pH sensitive oil entrapped calcium pectinate gel beads of acetoahydroxamic acid (Aha) was design in order to produce floating, gastric retentive and sustained drug release behaviour .

Floating drug delivery system and bioadhesive drug delivery are the widely used techniques for gastricretention[3-5]. Gastroretentive drug delivery systems like floating and bioadhesive drug delivery systems would improve the therapeutic effects of antimicrobial drugs. The bioadhesive drug delivery system can plug and seal the infected and inflamed mucosal cell lines adhered to the mucus layer, as well as to release the drug in a sustained manner[6]. *Helicobacter pylori (H. pylori)* are a small, spiral, microaerophilic, gram-negative bacteria and it is recognized to be associated with gastritis and duodenal ulcers. The microorganism has also been reported to be involved in the pathogenesis of other diseases, such as chronic atrophic gastritis, adenocarcinoma of the body or antrum of the stomach, gastro-esophageal reflux disease, peptic esophagitis etc[7] .

Acetohydroxamic acid is a small molecule, it can permeate intact bacterial cells and effectively inhibit the urease activity of *H. pylori.* Well diffusible Aha inhibits over 95% of urease activity after 10 min[8,9]. Pectin is colloidal polygalacturonic acid in galacturonic which some of the carboxylic group is esterified with methyl group .The main constituent of pectin is D galectouronic acid[10, 11]. The low methoxy polysaccharide, pectin, with the degree of esterification less than 50% can form rigid gels by the action of calcium ions or multivalent cations, which crosslink the galacturonic acid chains of pectin to yield hydro gels that are stable at low pH. Pectin can reduce interfacial tension between an oil phase and a water phase and is efficient for the preparation of emulsion[12]. In this study, the Aha loaded gastroretentive emulsion gel beads of calcium pectinate, were developed using the oils. The release behaviour of the gel beads capable of floating in gastric fluid was investigated with the aim to achieve a gastroretentive, controlled release formulation of Aha for its possible use as a carrier for oral delivery in the gastric region.

MATERIALS AND METHODS

Materials

Acetohydroxamic acid was purchased from Sigma-Aldrich Chemicals (India). Low methoxy pectin, calcium chloride, and ethyl cellulose were obtained from S.D. Fine Chem. India. India. Light mineral oil and castor oil were obtained from the Central Drug House, 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 calcium pectinate beads

The gel beads were formulated with 2^3 factorial design patterns. The effect of concentration of the oils (castor and mineral oils), pectin and calcium chloride were fixed in the formulation as independent variable. Effect of the dependent variables in the formulation was investigated in terms of bead diameter, floating lag time, encapsulation efficiency. The compositions of eighteen batches of the drug loaded in calcium pectinate beads were given in Table 1.

Table 1: Composition of drug loaded calcium pectinate gel beads.

Formulation code COB	MOB	Drug % w/v	Gum % w/v	Oil % w/v	Calcium chloride Mol ml^{-1}
D_1	R_1	0.75	1.25	05	0.275
D_2	R_2	0.75	1.25	10	0.275
D_3	R_3	0.75	1.85	05	0.275
D_4	R_4	0.75	1.25	05	0.450
D_5	R_5	0.75	1.25	15	0.275
D_6	R_6	0.75	1.85	05	0.450
D_7	R_7	0.75	1.25	15	0.450
D_8	R_8	0.75	1.85	10	0.450
D_9	R_9	0.75	1.85	15	0.450

COB = Castor oil entrapped formulation
HOB = Mineral oil entrapped formulation
Gum = pectin
Oil = mineral oil or castor oil.

Oil entrapped calcium pectinate gel beads were prepared by ionic gelation method. The drug was dispersed in varying concentrations of aqueous solution of pectin (1.25 -1.85 % w/v) with continuous stirring until uniform dispersion containing 0.75 w/v of the drug was obtained. The mixture was emulsified with either mineral oil or castor oil using Silverson emulsifier (Hicon, India) at a constant stirring rate of 500 rpm for 5 min. The resultant drug -loaded emulsions was dropped through a 21G syringe needle separately into 100 ml of 0.275- 0.45 mol ml^{-1} of calcium chloride ($CaCl_2$) solution, and stirred with a magnetic stirrer to improve the mechanical strength of the beads and to prevent their aggregation . The formation of small microbeads of acetohydroxamic acid based on either castor oil (COB) or mineral oil (MOB) occurred after 5 min of curing time. The beads were washed with distilled water, collected by filtration through whatman filter paper no. 1 and dried in tray dryer at 40°C for 6 hr.

Coating of gel beads

The Selected formulation beads were coated with ethylcellulose (EC) in 2^2 factorial design patterns for optimization (Table 2) .The coating substance was 5-10% (w/v) ethyl cellulose (EC) solution in acetone and times was 5 - 10 min . Gel beads (2 g) were placed in a fluidized bed dryer (TG 100, Retsch, Germany) and fluidized beads were sprayed with coating solution for period of 5 or 10 min at an air inlet speed of 220 m s^{-1} at room temperature. The beads were dried at room temperature for a period of 24 hr until all solvent was evaporated, leaving a film of ethylcellulose coat on the gel beads.

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

Formulation code	EC concentration % w/v	Time of coating (min)	% drug release t_{480} (min.) [a]	R^2
D_{61}	5	5	80± 1.2	0.943
D_{62}	5	10	77± 1.2	0.936
D_{63}	10	5	74± 1.2	0.918
D_{64}	10	10	70± 1.2	0.905

D_{61}, D_{62}, D_{63} and D_{64} = batch D_6 coated ethylcellulose coated optimize formulation batches.
R^2 = correlation coefficient, derived from zero order dissolution kinetic.
EC = ethyl cellulose
a = Mean ± SD (n = 3)

Morphology and size of microbeads

The particle size of the beads were determined in three sets using an optical microscope (Model BH-2, Olympus, Japan)fitted with a stage micrometer. Twenty dried beads were measured for the calculation of mean diameter. The external and internal morphology of the beads were studied by scanning electron microscopy (SEM). In this assessment, the beads were first coated with gold palladium under argon atmosphere using a gold sputter module in a high vacuum evaporator. The coated samples were then observed with SEM.

In vitro floating study

The *in vitro* floating study was performed using a USP 24 dissolution apparatus II having 500 ml of phthalate buffer solution (pH 3.4). The medium temperature was kept at 37 ± 0.5 °C. The floating beads (1.0 g beads) were soaked in the dissolution medium and the medium was agitated with a paddle at 50 rpm. After agitation, the beads that floated on the surface of the medium and those that settled down at the bottom of the flask were recovered separately. Lag time (the time taken for the beads to float at the surface of the medium) and floating behaviour was study up to 12 hr[13].

Determination of encapsulation efficiency and drug loading

Accurately weighed (100 mg) grounded powder of beads was soaked in 100 ml phosphate buffer (pH 7.5) and allowed to disintegrates completely for 4 hr [14]. The resulting dispersion was sonicated using a probe sonicator (UP 400 s, Dr. Hielscher GmbH, Germany) for 30 min and then filtered through a 0.45 μm filter. The polymeric debris was washed twice with fresh phosphate buffer to extract any adhered drug and drug content was determined spectrophotometrically at 502 nm against constructed calibration curve.

The drug content (DC) was calculated according to Eq 1.

$$DC\ \% = \frac{\text{weight of drug in beads}}{\text{weight of beads}} \times 100 \quad \ldots\ldots\ldots (1)$$

The encapsulation efficiency (EE) was calculated by Eq 2.

$$EE\ (\%) = (C/T) \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

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

In vitro drug release

In vitro dissolution studies were performed for all the formulation gel beads using USP 24 dissolution test apparatus II with a basket type [15]. An accurately weighed 50 mg amount of the beads were taken in to 900 ml dissolution medium of simulated gastric fluid (SGF, fasting state, pH 1.2) or fed state (phthalate buffer solution, pH 3.4) maintained at 37 ± 0.5 ºC and stirred at a speed of 50 rpm. At different time intervals over a period of 8 hr, a 10 ml aliquot of the medium was withdrawn and replenished with an equivalent volume of plain dissolution medium. The samples were filtered, suitably diluted and analyzed at a wavelength of 502 nm using a UV-visible spectrophotometer (Shimadzu). The drug release data were corrected for drug loss during sampling and degradation at acidic pH. All the tests were carried out in triplicate. Additionally, an experimental batch AE and AF containing 10 mg Aha and lactose (q.s.) filled in a capsule (# 2) was used as a reference formulation. Drug release data were corrected for the values of the drug loss during sampling.

Kinetic release evaluation

Kinetics of drug release from the micro gel bead, the release data were analyzed with various release kinetic models (, zero order, Higuchi and Korshmaer-Peppas) were applied to elucidate the mechanism of drug release from the beads in the fed state[15, 16, 17]. These Kinetic models were used to analysis of the dissolution study date with following equations (3) , (4) and (5) .

Zero-order model:

$$M_t = M_0 + K_0 t \quad \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Higuchi model:

$$M_t = M_0 + K_H t0{:}5 \quad \ldots\ldots\ldots\ldots\ldots\ldots (4)$$

Korshmaer-Peppas model:

$$M_t / M\infty = k\,(t)^n \quad \ldots\ldots\ldots\ldots\ldots\ldots (5)$$

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

Evaluation of concentration of the drug in gastric mucosa

The protocol of the study was approved by the animal ethical committee of the department. Albino rats were fasted for 8 hr and then divided into five groups , each of the group contains three animals. The animals were treated by an intraperitoneal injection of omeprazole at a 15 mg/kg dose to suppress gastric acid secretion. After one hour of omeprazole treatment, one group administered Aha (plain drug) and other groups were administered formulation batches ($D_{61,}$ D_{62}, D_{63}, and D_{64}) containing Aha equivalent dose of 40 mg/ kg . The rats were sacrificed at 1, 3 and 6 h after the administration. Then, the stomachs of the rates were removed and opened along the great curvature, the residue in the stomachs was removed carefully, and the stomachs were gently rinsed in 20 ml of distilled water and spread on a glass slide, the top layer was separated from the muscular layers. The removed mucosa was mixed with phthalate buffer (pH 3.4) in a glass tissue grinder and homogenate was centrifuged in a refrigerated ultracentrifuge at 3500 rpm for 5 min. The supernatant was removed and filtered through 0.45 µm filter. The amount of Aha contained in sample was measured by spectrophotometer method at 502 nm [18].

***In Vitro* Growth Inhibition Studies**

The bacterial strain used in this study was originally isolated with gastric biopsy from the peasant suffering with chronic gastritis and peptic ulcer in Institute of Medical Science, Banaras Hindu University Varanasi, India. Turbidimetric method with slight modification was employed to evaluate growth inhibition [19].The protocol of the study was approved by Institutional Animals Ethical Committee of the BHU. To suppress the growth of indigenous or exogenous contaminating bacteria, the isolated biopsy sample was grown in brucellla agar (Merck co Germany) containing 10 % horse blood, Vancomycine, polymyxine B and amphotericine and incubated at 37 ^{0} C for 7 d . The isolated sample was subcultured on Brucella agar contaning 10% hours blood without antibiotics and incubated at 37 ^{0} C for 3 d in microairofilic condition. *H. pylori* strain was grown in brucella broth at 37°C after 7 d in microaerobic atmosphere (5% O_2, 10 % CO_2, 85 % N_2). Growth of the bacteria was monitored by measuring the optical density (OD) of broth cultures with spectrophotometrically at λmax 640 nm[20].The numbers of bacteria were determined in terms of optical density by at a λmax *640* nm with one optical density unit corresponding to 10^8 colony-forming unit (CFU)/ml.To study the effect of formulations on *H. pylori* growth inhibition, 10 ml of nutrient broth containing *H.pylori* were transferred into sterile test tubes. Placebo bead (without drug), plain drug and optimized formulation of the ethylcellulose coated batches ($D_{61,}$ D_{62}, D_{63} ,and D_{64}) were taken containing Aha equivalent to 14 mmol l^{-1} which is four fold of the reported MIC_{50} for *H. pylori* urease [21] and added to the tubes and all the tubes were incubated at 37°C in a microaerobic atmosphere for 12 hr. The tubes containing culture were shaken at 100 rpm/min at 37°C in a microaerobic atmosphere condition in incubator for 12 hr. Then 100 µl of nutrient broth of *H.pylori* containing drug and different formulations were removed at various time points (3, 8 and 12 hr) and optical density was determined to assess growth inhibition of the bacteria by counting viable colony using spectrophotometer. The percentage growth inhibition was calculated using the following formula:

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

Where,

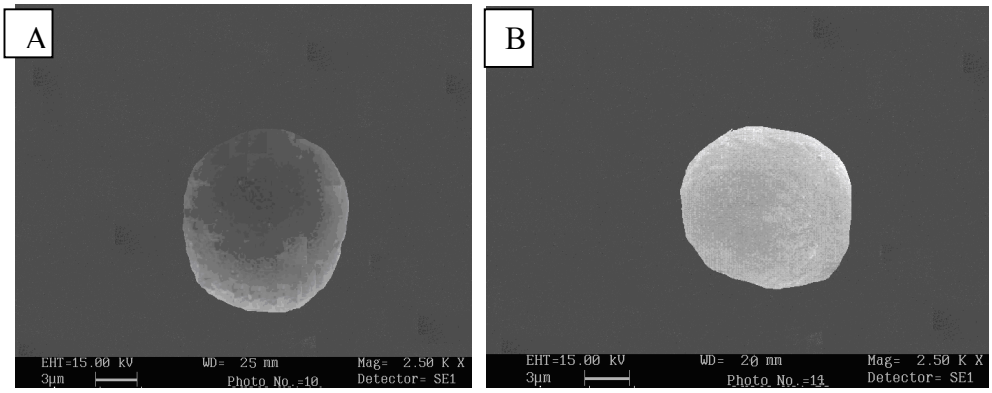
OD_{TP} = Optical density of test organism at particular interval.

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

Statistical analysis

The results were expressed as mean ± SD (standard deviation). Statistical evaluation of the data was performed using analysis of variance (ANOVA) and, depending on the outcome of ANOVA, Dunnett's multiple comparison tests were also applied. Statistically significant difference between the means of batches was set at $p < 0.05$.

Figure I: External structure under electron microscope of dried beads of (A) batch D_6 (COB) and (B) batch R_6 (MOB).



(A) (B)

RESULTS AND DISCUSSION

Gel micro beads were produce due to gelation and cross linking of calcium ions (Ca^{+2}), provided a gel barrier at the surface of the formulation. The cross linked Ca^{+2} are released in the acidic fluid of the stomach resulted to generation of in situ pores micro gel beads. The fluid front penetrate in the lattice of glassy polymer resulted in pH sensitive release of entrapped Aha in the stomach.

Morphology and size of microbeads

The Scanning electron micrographs (SEM) of the dried micro gel beads D_6 and R_6 are shown in Figure 1(A) and Figure 1(B). Gel beads prepared from mineral oil (MOB) are white, translucent and rigid, whereas castor oil entrapped gel beads(COB) were off -white translucent and elastic. The diameters of mineral oil entrapped formulation (MOB) varied 1.65±0.5 to 1.87±0.4 mm whereas castor oil gel beads (COB) varied 1.83±0.8 to 2.80±0.2 mm. Results revealed that beads were discrete, spherical in shape and smooth in tractors, this was due to presence of calcium ions (Ca^{+2}) , was contributed in homogenous bead formation. By increasing the oil concentration size of microgel beads increased and also presented that beads size was increased significantly ($P < 0.05$) an increased the polymer concentration, this could be attributed into increase in micro-viscosity of the polymeric dispersion eventually led in to formation of bigger beads. Large size beads were form if concentration of $CaCl_2$ increased, this inferences that excess Ca^{+2} were utilized three dimension cross-linking sites in the polymer resulting in mechanical stable larger gel beads formation.

In vitro floating study

Floating lag time was 14 -56 sec. and 22 to 80 sec for formulation MOB and COB , respectively . Floating ability of the microbeads may be explained as a result of penetration of the floating solution and entrapment of in situ generated carbon dioxide (CO_2) in to the bead matrix resulted in the swollen floating formulation. Increase in $CaCl_2$ beads resulted decrease in floating lag time due to increased in the porosity of the gel bead. Floating lag time was also rose as the concentration of the oils in the formulation increased and this can be attributed to flocculation of the oil globules which might also have coalesced to produce large droplets

Determination of encapsulation efficiency and drug loading

Encapsulation efficiency was highest for batch D_6 (79±0.3 %) and batch R_6 (73±0.5 %) while drug content was 72± 1.41 % and 47±1.25% in the respective for the batches . The effects of various formulation parameters on the drug encapsulation efficiency of formulated gel beads were shown in Table 3. The encapsulation efficiency of the beads rose as polymer concentration increased due to the availability of excess polymer which ensured that the drug was optimally entrapped. On the other hand, encapsulation efficiency decreased with increase in calcium chloride concentration because excess Ca^{+2} would have the effect of weakening the polymer gel structure and strength, thus leaving it more porous and limiting its capacity to trap the drug.

In vitro drug release

In vitro drug release study of the formulated beads was carrying out for 8 hr. The drug release from experimental gel beads (batch F) was 90 ± 1.48 % in empty state (pH 1.2) while that of fed state in Batch F_2 was found 88±1.45 % with in 3 hr in empty state pH 1.2 while at faded state (pH 3.4) condition, the release of the drug from the batch F_2 was 88±1.45 % in 3 hr of the study . The release of the drug from batch D_6 was 65 ± 1.24 % (fed condition) and from batch D_6E was 72± 1.23 % (empty condition) . The drug release from batch R_6 was 64± 2.73 % (fed condition) in empty state was 62± 2.73 % (R_6E) on 8 hr of the study (see Figure 3a). Drug release from the optimized formulations D_6 followed the Higuchi (R = 0.9241, n = 0.34) and Peppas models (R = 0.9427, n = 0.33). For the EC-coated beads (batch D_{61}), a maximum dissolution efficiency of 80± 1.2 % was attained in 8 hr, as shown in Figure 3 (b). The one-way analysis of variance (ANOVA) revealed a significant difference in vitro drug release profiles from the formulation batches of the fed and fasted states at a 95% confidence interval ($p < 0.05$) .The Release exponent (n) value suggested a diffusion-based release mechanism as the diffusion exponent values were less than 0.45[19]. However, the dissolution profiles of the coated beads (batch D_{61}, D_{62}, D_{63} and D_{64}) were best fitted to the zero-order kinetic model (Table 2).

Table 3 : Characterization of prepared microgel beads of calcium pectinate .

Formulation code		Diameter (mm)[b,c]		Lag time (sec)[c]		Encapsulation efficiency (%w/w)[c]		Drug content %[d, c]	
COB	**MOB**	**MOB**	**COB**	**MOB**	**COB**	**MOB**	**COB**	**MOB**	**COB**
D_1	R_1	1.65±0.5	1 .86±0.4	24±1.2	36± 1.4	61±0.2	64±0.4	45±1.23	57±2.24
D_2	R_2	1.73 ±0.4	1.83±0.8	32± 1.4	39±1.7	55±0.4	60±0.5	60±1.34	52±1.36
D_3	R_3	1.77±0.5	1.98±0.4	50±1.4	63±1.5	65±0.3	72±0.3	52±1.28	65±1.21
D_4	R_4	1.69±0.6	1.93±0.5	19±1.5	32 ±1.4	58±0.8	62±0.4	43±1.32	55±1.32
D_5	R_5	1.75±0.5	1.96±0.8	40±1.4	53± 1.3	63±0.7	54±0.5	55±1.36	47±1.34
D_6	R_6	1.87±0.7	2.08±0.8	14± 1.5	22±1.7	73±0.5	79±0.3	59±1.25	72±1.41
D_7	R_7	1.79±0.6	2.00±0.4	44±1.6	64±1.7	64±0.6	66±0.8	47±1.32	59±1.33
D_8	R_8	1.79±0.6	2.60±0.3	52±1.6	72±1.3	56±0.8	68±0.4	49±1.23	61±1.34
D_9	R_9	1.82±0.4	2.80±0.2	56±1.6	80±1.5	63± 0.7	66±0.9	47±1.33	59±1.26

COB = Castor oil used formulation, HOB = Mineral oil used formulation

a. = Mean ± SD (n = 3), b n = 20, c n = 3, d = Drug content in each100 mg of bead.

Formulation batch D61 was Considered the optimized gastroretentive controlled-release floating formulation of the study and efficient for targeted release of Aha in the stomach for cure of *H. pylori* infection.

Figure 3: Comparative drug release profile of the formulated batch (a) fasted and fed state condition and (b) in fed state ethyl cellulose coated batches.

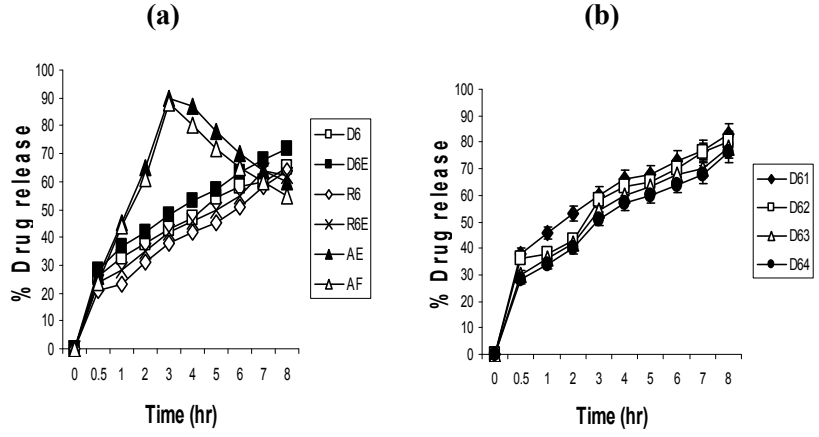
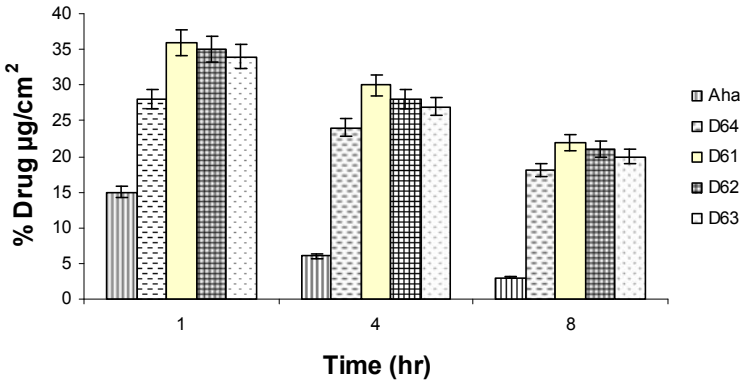


Figure 4: Drug concentration in gastric mucosa of the experi-mental animal treated with EC – coated formulation and batch Aha .



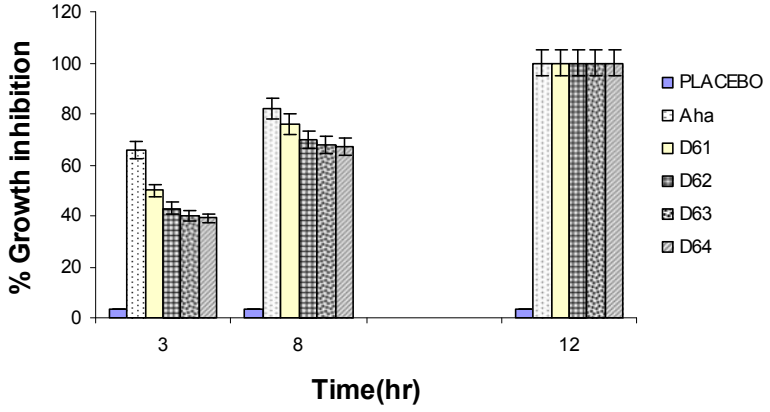
Evaluation of concentration of the drug in gastric mucosa

The drug concentration in gastric mucosa of the experimental animal was evaluated in order to assess availability of the drug in the vicinity of mucosal layer in the extended time. The drug was released from the microgel bead and attained initially concentration of 36±0.08 µg/ml (batch D_{61}), 35±0.02 µg/ml (batch D_{62}), 34±0.05 µg/ml (batch D_{63}), 28±0.06 µg/ml (batch D_{64}), and 15±0.06 µg/ml (batch Aha) after one hour of the drug administration and the concentration was decreased to 22 ±0.02 µg/ml, 21±0.04 µg/ml , 20±0.08 µg/ml, 18±0.05 µg/ml and 3±0.03 µg/ml , respectively at 8 hr (Figure 4) of the treatment .By administration of batch $D_{61,}$ higher concentration of the drug attained initially, this may be due to easily partition of the drug from the formulation barrier in to the gastric mucosa.

***In vitro* growth inhibition studies**

Percentage growth inhibition of the microgel beads against *H. pylori* was evaluated for placebo, plain Aha and EC coated formulation (batch D_{61}, batch D_{62}, batch D_{63} and batch D_{64}). *In-vitro* growth inhibition of *H. pylori* was investigated at various time intervals up to 12 hr. The antimicrobial efficacy of formulations and free Aha was determined in terms of percentage growth inhibition. The *H. pylori* culture tubes containing placebo microbeads did not show significant growth inhibition (3.3 ±1.2 %) at the end of 12 hr of the incubation (Figure 5). The percentage growth inhibition of the plain Aha was 82±1.6 % and EC coated formulation was showed growth inhibition varied 76 ±1.5 % (batch D_{61}) > 70 ±1.4 % (batch D_{62}) >68 ±1.4 % (batch D_{63}) >67 ±1.4 % (batch D_{64}) in 8 hr of the study . The results clearly indicated that optimized formulation (batch $S_{41)}$ was more effective compare to the other; this could be due to interaction and adsorption of the Aha more extensively compare to the others batch . Hence, it can be expected that the batch D_{61} may abolish all the mechanisms of the bacterial survival *in vivo* and may provided evidence for optimized batch of the study which is significantly cure the microbial infection.

Figure 5: Percentage growth inhibition of *H. pylori* for the prepared microgel beads.



CONCLUSION

The designed therapeutically efficacious oil-entrapped gel beads were excellent system for floating controlled drug release for long time through out acidic pH of gastric region. This property of the formulation applicable to devoid of disadvantage of single unit dosage form due to the advantage of gastroretantive excellent buoyancy in term of sustained drug release over several hours. This property help full to provide effective deliver of acetohydroxamic acid at stomach site and may be use as effective pH trigger vehicle that may open new option for targeting the acetohydroxamic in the stomach for *H pylori* eradication.

ACKNOWLEDGEMENT

Authors are thankful to Dr. P.R. Mishra, the Scientist in Pharmaceutics Division, Central Drug Research Institute Lucknow, India for his valuable support for this research. Special thanks also to Director, Saroj Institute of Technology and Management Lucknow for providing facilities to carry out the study.

CONFLICT OF INTEREST

The authors associated with the study have strictly declared that they have no conflict of interest**.**

CONTRIBUTION OF AUTHORS

We declare that this work was done by the authors (Girish Kumar Tripathi and Satyawan Singh) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors .Mr. Girish Kumar Tripathi was conceived and design protocol of the study and further the study was improved by Dr. Satyawan Singh .The Tripathi was also involved in analysis of the study data.

REFERENCES

1. Rouge N. , Buri P., Doelker E., Morphology and buoyancy of oil-entrapped calcium pectinate gel beads, Int. J. Pharm., 1996, 136, 117–139.
2. Ichikawa M, Watanabe S, Miyake Y. , A new multiple unit oral floating dosage system: Preparation and *in vitro* evaluation of floating and sustained-release kinetics , J. Pharm Sci., 1991, 80, 1062-1066.
3. Choi B.Y., Park H. J. , Hwang S. J. , Park J. B. , Preparation of alginate beads for floating drug delivery system: effects of CO_2 gas-forming agents , Int. J. Pharm ., 2002 , 239 , 81-91.
4. Nagahara N., Akiyama Y., Nako M. , Tada M., Kitano M., Mucoadhesive Microspheres Containing Amoxicillin for Clearance of *Helicobacter pylori* , Antimicrob. Agent. Chemother., 1998 , 42, 2492–2494.
5. Cooreman M.P., Krausgrill P., Hengels K.J. , Local gastric and serum amoxicillin concentrations after different oral application forms, Antimicrob. Agents Chemother., 1993 , 37 , 1506–1509.
6. Yeole P.G. , Khan S., Patel V.F., Floating drug delivery system: Need and development , Indi. J .Pharm Sci . , 2005 , 67 , 265–272.
7. Suerbauma S. , and Michetti P. , *Helicobacter pylori* infection , E. Engl. J. Med . , 2002 , 347 , 1175-86.
8. Phadnis S. H. , Parlow M. H. , Levy M. , Iher D. , Canldine C. M. , Connors J. B. , Dunn B.E., Surface localization of *Helicobacter pylori* urease and a heat shock protein homolog requires bacterial autolysis. Infect Immun , 1996 , 64, 905–912.
9. Park K. and Robinson J.R. , Bioadhesive polymers as platforms for oral controlled drug delivery: methods to study bioadhesion , Int . J. Pharm. , 19884 , 19 , 107-127.
10. Rolin C., Whistler R.L., Bemiller J.N. , Industrial Gums: Polysaccharides and their derivatives. New York, Academic Press , 1993 , 257-293.
11. Schols H. A. and Voragen A.G. , Complex pectin: structure elucidation using enzymes. In: Visser J, Voragen AGJ, eds. Progress in Biotechnology: Pectin and Pectinases. Amsterdam, the Netherlands, Elsevier , 1996 , 3-19.
12. Leroux J. , Langendorff V. , Schick G. , Vaishnav V. , Emulsion stabilizing properties of pectin. Food Hydrocolloids . , 2003 , 17 , 455-462.
13. Cooreman M. P. , Krausgrill P. , Hengels K.J. , Local gastric and serum amoxicillin concentrations after different oral application forms , Antimicrob Agents Chemotherm. , 1998 , 37 , 1506–1509.
14. United State Pharmacopeia , 24 /National Formulary 19, USP Convention. Rockville , MD.2002.
15. Wagner J. G . , Interpretation of percent dissolved-time plots derived from in vitro testing of conventional tablets and capsules, J. Pharm Sci .1969, 58,1253–1257.
16. Higuchi T. , Mechanism of sustained-action medication: theoretical analysis of rate of release of solid drug dispersed in solid matrices , J . Pharm Sci. , 1963 , 52 , 1145–1149.

17. Korsmeyer R.W., Gurny R., Peppas N., Mechanisms of solute release from porous hydrophilic polymers , Int. J. Pharm. 1983 , 24, 25–35.
18. Westblem T.U. , Duriex D. E., E. Madan E. , Guinea pig model for antibiotic transport across gastric mucosa: inhibitory tissue concentrations of clindamycin against Helicobacter pylori (Campylobacter pylori) following two separate dose regimens , Antimicrob. Agents Chemother. , 1990, 34: 25-28.
19. N. Portal, M. Glaser, Saraga E. , Oral immunization against *H. felis* infection , Gastroenterol., 1995, 107: 1002–1011.
20. Umamaheswari R.B. , Jain S,Tripathi P.K. , Floating-Bioadhesive Microspheres Containing Acetohydroxamic Acid for Clearance of *Helicobacter Pylori* , Drug Deliv. , 2002, 9 , 223-231.
21. Mobley H.L., Hu L.T. , Foxall P. , *Helicobacter pylori* urease: Properties and role in pathogenesis , J. Gastroenterol. , 1991, 187 , 39–46.
22. Streubel A. , Siepmann J. , Bodmeier R. , Floating microparticles based on low density foam powder , Int. J .Pharm. , 2002, 241, 279–292.

*****