Floating-Mucoadhesive Beads of Clarithromycin for the Treatment of *Helicobacter pylori* Infection

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An objective of the present study was to develop alginate/hydroxypropyl methylcellulose (HPMC) based floating-mucoadhesive beads of clarithromycin to provide prolonged contact time of antibiotic to treat stomach ulcer. Floating-mucoadhesive beads were prepared and characterized for *in vitro* performance followed by investigation of *ex vivo* study in albino-wistar rats. Beads were prepared by ionic gelation technique where calcium chloride used as gelating agent and incorporated liquid paraffin for floating of the beads. Prepared beads were evaluated extensively for particle size, drug entrapment; swelling and surface morphology by using scanning electron microscopy. X-ray radioimaging study in rabbits, *in vitro* mucoadhesion using rat stomach mucosal membrane and *in vitro* drug release studies were carried out. *Ex vivo* performance of alginate-HPMC beads were studied using albino rats in comparison to simple alginate-calcium beads. Alginate-HPMC beads may be suitable floating-muco-adhesive drug delivery system for delivering clarithromycin to treat stomach ulcers.

Key words beads; alginate hydroxypropyl methylcellulose; liquid paraffin; floating-mucoadhesion; clarithromycin; stomach ulcer

Helicobacter pylorus (*H. pylori*) is a gram-negative, motile, microaerophilic spiral bacterium. Helicobacter species is the causative organism for peptic ulcer. Gastric (mainly peptic) ulcer is one of the major ailments affecting about 60% of human adults and nearly 80% of child population, all *H. pylori* species cause some degree of persistent inflammation.[1] Treatment of *H. pylori* remain a challenge although *H. pylori* is highly sensitive to most antibiotics, eradication of *H. pylori* from patient is difficult, even with the current best therapies.[2,3] Conventional tablets and capsules are used for eradication therapy; do not remain in stomach for a long time.[4] The various floating preparations such as micro balloons, granules, powders, capsules, tablets, and laminated films has been attempted.[5] An excellent concept of floating system suffers from a disadvantage that it is effective only when the fluid level in stomach is sufficiently high; however, as the stomach empties and the tablet is at the pylorus, the buoyancy of the dosage form may be impeded.[6] This serious limitation can be overcome by the use of bio-adhesive polymers to enable it to adhere to the mucous lining of stomach wall. Floating and bio-adhesive drug delivery systems offer the advantages of increased contact time with stomach mucosa, more effective absorption and bioavailability of drugs with absorption windows near proximal intestine and stomach, and low dosing frequencies.[7] In this article a synergism between bio-adhesion and floating property has been explored. Therefore, it is difficult to reach minimum inhibitory concentration in the gastric mucosa where *H. pylori* colonizes. To overcome these problems, a new concept is proposed based on a floating and bio-adhesive system with site-specific drug delivery.[8] 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.

Clarithromycin is the drug of choice to treat peptic ulcer as *H. pylori* resistance rate is much lower for clarithromycin as compared to other antibiotics like amoxicillin and tetracycline. Stomach-specific antibiotic drug delivery would be highly beneficial in the treatment of *H. pylori* infection in peptic ulcer disease.[9] Clarithromycin is having good stability in a gastric pH.[10] The low density hydroxypropyl methylcellulose (HPMC) polymers have good bio-adhesive property. Polysaccharides like chitosan and gum acacia was found to float but not much promising like oils.[11] Thus incorporation of oil in the drug delivery system with the Alg-HPMC beads shows good floating and muco-adhesion property.[12]

The present work describes incorporation of an active component known to be effective against *H. pylori*, clarithromycin, into alginate (Alg)-Ca and Alg-HPMC beads and reports the release of drug from the beads in an acidic environment and also applies the new approach of mucoadhesive and floating for synergistic effect.

Experimental

Materials Clarithromycin and HPMC K4M were obtained as a gift sample from Biochem Pharmaceuticals (Daman, India) and Colorcon Asia Pvt. Ltd. (Goa, India) respectively. Sodium alginate, calcium chloride and light liquid paraffin were supplied by Loba Chemie Pvt., Ltd. (Mumbai, India). All other ingredients used were of analytical grade.

Preparation of Alginate Gel Bead Containing Liquid Paraffin (Alginate-Ca) Sodium alginate (4% w/w) was dissolved in 50 ml of distilled water with agitation. Clarithromycin (CL) and liquid paraffin (LP) were added to the above solution. Prepared solution containing CL (250 mg) and LP (5/10/15% w/w) was added drop-wise into 100 ml calcium chloride (2% w/v) and left at room temperature for 2 h. The resultant hydrogel beads were washed twice with 50 ml of distilled water and dried at room temperature for

Table 1. Formulation Composition, % EE and % Drug Loading

Formulation code	% Na alginate	% HPMC K4M	% Liquid paraffin	% Encapsulation efficiency	% Drug loading
P1	3	—	0	82.53	29.61
P2	4	—	0	88.65	30.65
P3	4	—	5	83.81	31.44
P4	4	—	10	87.74	37.03
P5	4	—	15	88.90	34.24
P6	3	0.5	0	85.48	28.21
P7	3	0.5	5	86.44	35.03
P8	3	0.5	10	86.58	29.49
P9	3	0.5	15	83.26	23.48
P10	3	0.5	20	81.66	22.41

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24 h (Table 1).

Preparation of Alginate Gel Bead Containing HPMC K4M and Liquid Paraffin (Alginate-HPMC) Sodium alginate (3% w/w) was dissolved in 50 ml of distilled water with agitation and HPMC K4M was added with slow stirring. Simultaneously CL (250 mg) and LP (5/10/15/20% w/w) were added to the above solution. Solution containing CL and LP was added drop-wise into 100 ml calcium chloride (2% w/v) and left at room temperature for 2 h. The resultant hydrogel beads were washed twice with 50 ml of distilled water and dried at room temperature for 24 h (Table 1). As CL is insoluble in LP, both exist in gel beads.

Characterization of the Beads. Size Analysis Alginate calcium (Alg-Ca) and Alg-HPMC containing CL beads were evaluated for particle size. The bead sizes ($n=50$) were taken for particle size analysis and average particle size was determined. Particle size of the beads was measured using a Motic DMWB2-223 digital microscope (Canada) fitted with 1/3 CCD Camera Imaging accessory and using Motic Images 2000 (1.3 Version) image analysis software.

Morphological Analysis Surface and cross-sectional morphologies of beads were examined with the Scanning Electron Microscope (SEM) (JSM-5310LV, Jeol, Japan).

Beads were mounted on metal grids using double-sided tape and gold coated under vacuum.

Buoyancy Study Buoyancy test was carried out using USPXXII dissolution test apparatus. Each dissolution jar was filled with 0.1 M HCl solution (pH 1.2, 900 ml) and maintained at temperature 37±0.5°C. Fifty beads were added in each jar and stirred with paddle at 50 rpm for 10 h. Beads remaining buoyant in 0.1 M HCl solution are observed visually and calculated the % buoyancy.

Drug Loading and Encapsulation Efficiency (EE) The beads (25 mg) loaded with CL were added in 0.1 M HCl solution (pH 1.2) under stirring. The mixture was filtered with the Whatman filter paper and the amount of CL was determined spectrophotometrically at 485 nm. The concentration in the sample was used to calculate the loading by dividing the weight of beads initially added. The CL encapsulation efficiency was estimated according to the formula.[13]

$$\text{encapsulation efficiency (\%)} = \frac{M_{\text{actual}} \times 100}{M_{\text{theoretical}}} \quad (1)$$

Swelling and Erosion Study Swelling behavior of beads can be determined by their water absorbing capacity. Swelling study was done by using USP XXII dissolution test apparatus.[14] Two-fifty milligrams of the dry beads kept in muslin cloth and tied to the lower end of the paddle and immersed in acid buffer (pH 1.2). One and 2 h time interval beads were withdrawn from muslin cloth and wiped gently with tissue paper and weighted. Same procedure was continued up to 10 h to calculate percentage erosion. After 10 h beads were separated from the medium using stainless steel grid, wiped gently and dried for 24 h at 30 °C and weighted. The percentage swelling and erosion were calculated according to the Eqs. 2 and 3.

$$\%\ \text{swelling} = \frac{\text{weight of swollen bead} - \text{initial weight of the beads}}{\text{initial weight of beads}} \times 100 \quad (2)$$

$$\%\ \text{erosion} = \frac{\text{weight of beads after drying}}{\text{initial weight of beads}} \times 100 \quad (3)$$

Drug-Excipients Compatibility Study. FT-IR Spectroscopy The crushed beads were mixed with potassium bromide (Merck) in 1 : 100 proportions and dried at 40 °C. The mixture was compressed to a 12 mm semi transparent disk by applying a pressure of 10 tons (KBr press, tsi, Mumbai) for 2 min. The FT-IR spectra over the wavelength range 4000—400 cm^{-1} were recorded using a FT-IR spectrometer (FTIR-8400S, Shimadzu, Japan).[15]

Differential Scanning Calorimetry (DSC) Analysis DSC (Perkin Elmer Cyris-DSC, U.S.A.) was performed on CL (A) and CL loaded beads (B).The thermograms of samples were obtained at a scanning rate of 10 °C/min conducted over range of 30—300 °C.

***In Vitro* Drug Release Study** The drug release profiles of CL-loaded beads were carried out in 0.1 M HCl solution (pH 1.2, 900 ml) at 37±0.5 °C using a USP XXII dissolution test apparatus (Electrolab, EDT08, India, paddle method). The rotation speed of the paddle was adjusted to 100 rpm for homogenous dispersion of gel beads in dissolution medium. An aliquot of 5 ml was withdrawn periodically from test solution and replaced with 5 ml of fresh medium maintained at 37±0.5 °C and assayed spectrophotometrically at 485 nm (UV-1700, Shimadzu, Japan). All drug release profiles were performed in triplicate. In order to investigate the drug release mechanism, the release data were fitted to models representation zero order, first-order, Higuchi and Peppas–Korsemeyer equation.[16,17]

Drug Release Kinetics The kinetic modeling of release data was done using above equations. In spherical matrices, if $n \leq 0.43$, a Fickian diffusion (case-I), $0.43 \leq n < 0.85$, a non-Fickian transport and $n \geq 0.85$, a case-II transport (zero order) drug release mechanism dominates.[18,19]

***In Vitro* Mucoadhesion Studies** *In vitro* mucoadhesion studies were carried out using rat stomach mucosa by the reported method with necessary modifications.[20] Overnight fasted male wistar rats (200—250 g) were sacrificed and stomach mucosa was excised and washed with physiological saline at the rate (5 to 10 ml/min for 10 min, then 20 to 30 ml/min for *ca.* 20 min) by using peristaltic pump. About 500 ml of physiological saline was used for cleaning the mucosa. After 15 min the mucosa was held in inclined position (Fig. 1). Mucosa was fixed to the glass slide with the cynoacrylate glue and about 50 beads (N_0) hydrated with little amount of water and dispersed on the mucosal tissue and left on it for 20 min for the interaction with the mucosal surface. During this period whole system was placed in a constant humidity chamber which was adjusted to 90% relative humidity. At the end, the system was washed with 0.1 M HCl solution (pH 1.2) at the rate of 22 ml/min using a peristaltic pump. After 20 min beads detached from the mucosa (N_s) were observed visually and percent mucoadhesion was calculated by the following equation.[21,22]

$$\%\ \text{mucoadhesive strength} = \frac{N_0 - N_s}{N_0} \times 100 \quad (4)$$

***Ex-Vivo* Mucoadhesion Study of Beads** The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) of R. C. Patel College of Pharmacy, Shirpur and is in accordance with guidance of Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Six albino-wistar rats were fasted overnight. Optimized formulation (P8) was administered to rats by using rat feeding needle. Rats were kept fasted until they were sacrificed, respectively, in 0, 1, 2, 5, 7 and 10 h. The beads remained in the stomach were counted, and the % adhesion calculated according to the equation.[23]

***In Vivo* X-ray Imaging Study** In order to check the actual floating ability of the beads on the gastric content and its usefulness in achieving a sustained release, the *in vivo* floating time (FT) and the gastric transit time (GRT) of the floating system were determined. The *in vivo* study was carried out by administering floating beads to rabbits and monitoring them through a radiological method. Three adult male New Zealand white strain rabbits weighing approximately 2—2.5 kg were used for the study. After fasting for 24 h, rabbits were allowed free access for food pellets and water for 12 h. Optimized floating beads were obtained by adding 50% of X-ray grade barium sulfate (this amount was determined experimentally to allow X-ray visibility but not to shun floatation of the beads). Fifty milligrams of beads containing 50% of barium sulfate were administered through a gastric tube with the aid of 3—4 ml of water, using a syringe to push the beads forward. The position of floating beads was monitored by X-ray photographs after confirmation of gastrointestinal tract (GIT).

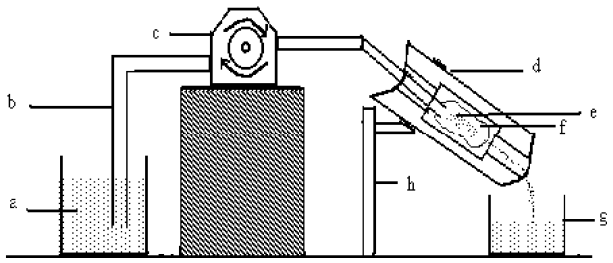

Fig. 1. Schematic Diagram of the Model Used to Test the Mucoadhesion of Beads

(a) Reservoir containing the 0.1 M HCl (pH 1.2); (b) polypropylene tube; (c) peristaltic pump; (d) polypropylene plastic support; (e) stomach mucosa; (f) beads; (g) reservoir for collecting the washing; (h) stand.

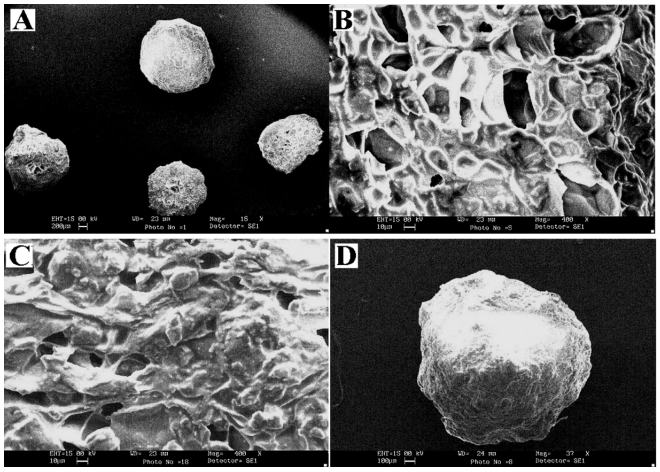

Fig. 2. Scanning Electron Micrographs of CL Floating-Mucoadhesive Beads (P7)

(A) Upper view of beads, (B) cross section, (C) surface of beads, and (D) intact appearance of beads.

Results and Discussion

Microscopical Characteristics of Beads Microscopical characteristic of beads indicate that the Alg-Ca beads having the size between 0.7 to 1 mm and Alg-HPMC beads having the size 0.8 to 1.1 mm. Study shows that, as the amount of oil increases, bead size also increases.

Morphological Analysis Careful examination of representative beads of each formulation using SEM microscopy revealed more detail information regarding their external and internal morphological features. As it can be seen from the SEM images (Fig. 2) the beads presented a rough surface with characteristic large wrinkles and micropores.The presence of HPMC has not any impact on the external morphology of the beads and no specific localizations of the HPMC or CL were observed in the matrix.

Buoyancy Study The floating ability of the prepared formulations was evaluated in 0.1 M HCl solution (pH 1.2) using a USP XXII dissolution test apparatus at 100 rpm. The percentage of floating beads on the dissolution medium surface was evaluated (data not shown). Results shows that formulations P4, P5, P8, P9 and P10 containing LP shows 100% buoyancy and formulation P1, P2 and P6 without LP shows 0% buoyancy. Floating ability of beads depends upon the percentage of LP used in the preparation of beads. The floatation of beads was observed due to the entrapment of LP in gel matrix.

Drug Loading and Encapsulation Efficiency Drug loading capacity of Alg-Ca beads was found to be 29.61 to 34.24% and encapsulation efficiency was 82.53 to 88.90%. In case of Alg-HPMC 22.41 to 35.03% and encapsulation efficiency was 81.66 to 86.58%. Drug loading and encapsulation efficiency are shown in Table 1. There was no considerable effect of liquid paraffin found on drug loading and encapsulation efficiency of CL. The percentage efficiency was high because bead formation was carried out in distilled water, in which CL is insoluble, with a lesser possibility of leaching of the drug during encapsulation.[24)]

FT-IR Spectroscopy CL, Alg-Ca beads, CL loaded Alg-Ca beads, Alg-HPMC beads and CL loaded Alg-HPMC beads were studied for FT-IR. Drug characteristics such as –CH– stretch (2929.6 cm^{-1}), C=O stretch (1635.53 cm^{-1}), C–N bend (1278.72 cm^{-1}), C–OH, stretch (3384.84 cm^{-1}). The characteristic peak of CL was not altered after encapsulation, indicating no chemical interaction between drug and polymer (data not shown).

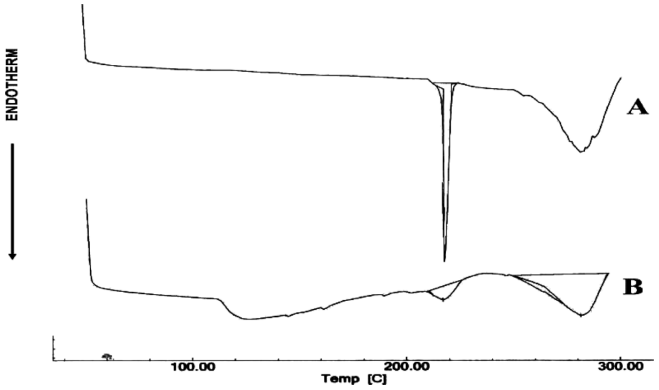

Fig. 3. DSC Thermogram of CL (A) and CL Loaded Beads (B)

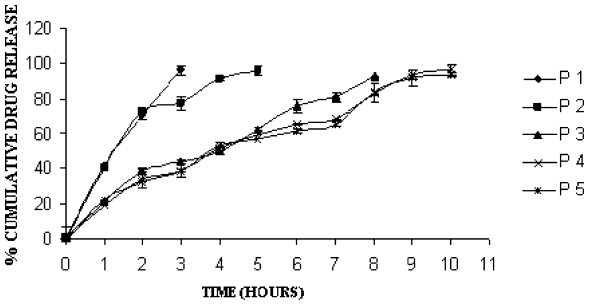

Fig. 4. *In Vitro* Dissolution of CL from Alginate-Ca Floating-Mucoadhesive Beads of Formulation P1, P2, P3, P4 and P5

DSC Study The DSC thermogram (A) showed a sharp endothermic peak at 220 °C for pure CL as the melting point of the drug, while in the DSC thermogram of the drug-loaded beads (B), the endothermic peak was observed at 221.96. Reduction in the sharp intensity of endothermic peak of drug-loaded beads revealed molecular dispersion of drug in the matrix. The results are shown in Fig. 3.

Swelling and Erosion Study of the Beads Swelling of the beads was measured on the basis of the water absorbing capacity. Formulation P6 and P7 showed higher swelling might be due to HPMC and less concentration of LP (0, 5%) respectively. Formulation P3, P4, P5 and P8, P9 and P10 showed less swelling. This might be due to two factors 1) HPMC and 2) concentration of LP. Formulation P3, P4, P5 contains only sodium alginate and formulation P8, P9 and P10 contains HPMC and LP. Absence of HPMC in the formulation leads to less swelling and higher concentration of LP affects the swelling. The loss of weight of beads measured as % erosion. The rate of erosion is proportional to the amount of LP and HPMC. Formulation P3, P4, P5 contains sodium alginate, shows fast gelling and fast erosion. In case of formulation P8, P9 and P10 contain HPMC and higher concentration of LP (10, 15, 20% respectively). HPMC increases the swelling of beads and LP decreases the swelling.

***In Vitro* Drug Release Study** Alg-Ca beads (P1, P2) show rapid release of CL within 3—5 h, no more being released after 5 h. As the concentration of LP increases from

Table 2. Data of Release Kinetics Parameters

Formulation	Zero order	First order	Higuchi	Peppas	*n* value
P1	0.9872	0.8946	0.9730	1.0000	0.7672
P2	0.8668	0.9797	0.9799	0.9323	0.5237
P3	0.9733	0.9029	0.9640	0.9794	0.6977
P4	0.9758	0.8660	0.9672	0.9900	0.6900
P5	0.9662	0.8915	0.9634	0.9803	0.6312
P6	0.9574	0.8991	0.9749	0.9754	0.7411
P7	0.9808	0.8830	0.9661	0.9829	0.6550
P8	0.9770	0.8776	0.9704	0.9873	0.6608
P9	0.9808	0.9241	0.9704	0.9873	0.6608
P10	0.9419	0.9452	0.9763	0.9750	0.5347

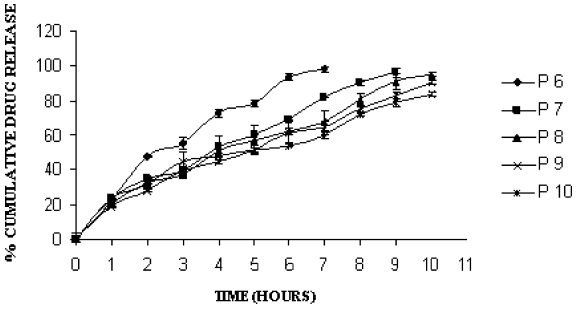

Fig. 5. *In Vitro* Dissolution of CL from Alg-HPMC Floating-Mucoadhesive Beads of Formulation P6, P7, P8, P9, and P10

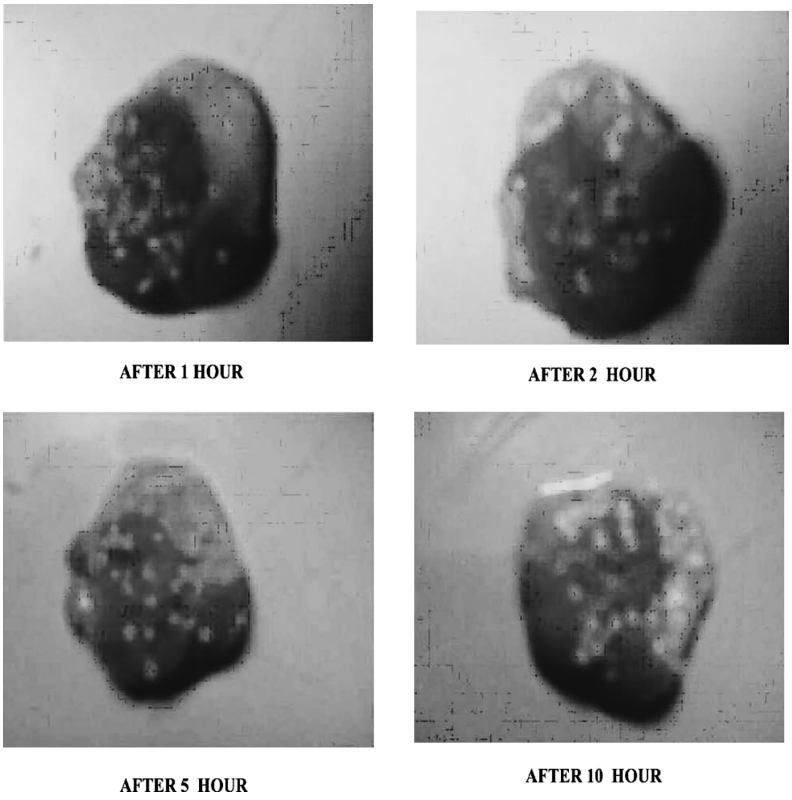

Fig. 6. *Ex Vivo* Study of Alg-HPMC (P8) Beads

5—15%, retards the release of CL from beads. P3 retards the release up to 8 h and P4, P5 up to 10 h. Alg-HPMC (P6, P7) show release of CL up to 7—9 h. Formulation P8, P9, P10 shows 95, 90 and 83.76% respectively and retards the drug release up to 10 h. Formulation P4 and P8 were optimized on the basis of *in vitro* drug release, percent mucoadhesion and kinetic release. Authors found that formulation P8 is suitable for stomach specific delivery of CL by floating and mucoadhesion for 10 h. Formulation P4 shows less mucoadhesion than P8 but it sustains the drug release for 10 h without HPMC as shown in Figs. 4 and 5. Murata *et al.* reported that the release rate decreased with increase in the amount of olive oil.[25] LP produces hardening to the hydrogel beads. As the concentration of LP increases from 5—20%, retards the release of CL from beads up to 9—10 h. HPMC also plays role in retarding the drug release which has been shown in Figs. 4 and 5.

Drug Release Kinetics Based on these estimations the fit of each model was predicted. Considering the R^2 values, the calculated Peppas model[26,27] successfully fitted the Alg-HPMC formulations. An anomalous (non-Fickian) mechanism became apparent for all formulations. However, the addition of HPMC increased the diffusivity of CL in the matrix in the medium without altering the release mechanism. All the other models such as first order,[28] Higuchi were not able to fit the CL release profiles. As it can be seen in Table 2 there are some formulations where the Higuchi also fitted the release profiles. In this case the selection of an adequate model was based on comparisons of the following features of each model: higher determination coefficient; smaller standard error of model parameters; and smaller residual mean square. On the basis of these comparisons, Peppas model fitted best for all release profiles respectively. Results are summarized in Table 2.

***In Vitro* Mucoadhesion Study** Mucoadhesion study was done by flow through cell as shown in the Fig. 1. Alg-Ca

Table 3. *Ex Vivo* Study of Formulation P4 and P8

Formulation code	Beads adhered to stomach mucosa after time *T*						% Adhesion of beads after 10 h
Time (h)	0	1	2	5	7	10	
P4	25	25	21	16	14	8	32%
P8	25	25	23	21	18	18	72%

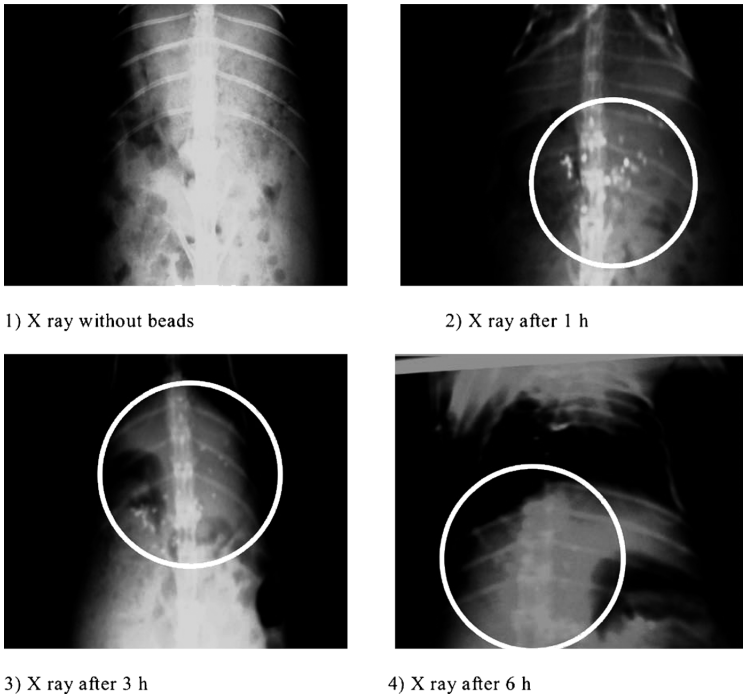

Fig. 7. X-ray Photographs of Rabbit Stomach without Beads (1), after 1 h (2), after 3 h (3), after 6 h (4)

beads having the mucoadhesion up to 80% and no significant effect of the LP on the mucoadhesive property of the beads. Alg-HPMC beads such as P8, P9, and P10 showed 100% mucoadhesion. Result of *in vitro* mucoadhesion study shows that, HPMC increases the considerable mucoadhesive property of the beads and there was no effect of the LP on the mucoadhesive property of the beads.

***Ex-Vivo* Mucoadhesion Study** Alg-HPMC beads (P8) were administered orally to albino-wistar rats under fasted conditions. The mucoadhesiveness of the beads in the stomach were studied. The mucoadhesion of Alg-HPMC beads at 1, 2, 5, 7 and 10 h after administration was shown in Fig. 6 and Table 3. *Ex-vivo* mucoadhesion study shows floating-mucoadhesive beads of Alg-HPMC have better adhesive effect in the stomach and might stay longer in the stomach for more effective *H. pylori* clearance.

***In Vivo* X-ray Imaging Study** Alg-HPMC beads (P8) formulation showed good mucoadhesion *in vitro* and *ex vivo* mucoadhesion study. Same optimized formulation is studied for *in vivo* X-ray imaging study. Only the Alg-HPMC mucoadhesive floating beads were used for *in vivo* X-ray imaging study. In this study, monitoring portion was confirmed by the administering barium sulfate to the fasted animals and images have been taken after complete saturation of GIT (Image 1). Images 2—4 were taken immediately after 1, 3 and 6 h respectively. The presence of beads in stomach can be clearly noticed that, the bead remains in the stomach not being subjected to disintegration in all rabbits. *In vivo* X-ray imaging study clearly indicate that the prepared beads of CL remained buoyant for at least 6 h in rabbit stomach and that they had good floatability *in vivo*. X-ray photographs are shown in Fig. 7.

Overall, the results indicate that optimized formulation shows 60% drug concentration for 6 h in stomach where it is needed for local action (with reference to *in vitro* release data and *in vivo* X-ray imaging study).

Conclusion

Alg-HPMC beads may be more suitable floating-mucoadhesive drug delivery system for delivering CL to treat stomach ulcers compared with Alg-Ca.

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