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Formulation and *In vitro* evaluation of pH sensitive oil entrapped polymeric blended buoyant beads of Amoxicillin

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Abstract

*The design of effective drug delivery systems has recently become an integral part of the development of new medicines. The objective of this study was to develop oil entrapped buoyant bead of calcium pectinate blended hydroxypropyl methyl cellulose (HPMC) or Carbopol 934P in order to evaluate its potential in the targeted sustained delivery of amoxicillin in the gastric region. The formulation was prepared by inotropic gelation technique using sodium bicarbonate as gas forming agent and formulation was emulsified with mineral oil. The gel beads were evaluated in term of bead diameter, floating %, encapsulation efficiency and in vitro drug release. The drug release was demonstrated as sustained pattern for 8 h, which was best fitted in the Peppas model with $n < 0.45$. Formulation of the batch was coated with ethyl cellulose (EC). Optimized coated microbeads were exhibited zero-order sustained pattern of the drug release up to 8 h. Hence prepared gel bead may be used to incorporate antibiotics like amoxicillin and may be effective when administered locally in the stomach against microbial infection like *H. pylori*.*

Key words: Amoxicillin, pH sensitive drug delivery system, Hydrophilic polymer, Buoyancy.

INTRODUCTION

Despite tremendous advancement in drug delivery, oral route of administration has received the more attention and success because the gastrointestinal physiology offers more flexibility in dosage form design than other routes. Hence, research continuously keeps on searching for ways to deliver drugs over an extended period of time, with a well-controlled release profile. Gastric emptying of dosage form is extremely variable process and ability to prolong and control the emptying time. Gastric transit time is valuable asset for dosage forms, which reside in the stomach for a long period of time than conventional dosage form. Conventional oral dosage forms such as tablets, capsules provide specific drug concentration in systemic circulation without offering any control over drug delivery and also cause great fluctuations in plasma drug levels. Many attempts have been made to develop sustained release preparations with extended clinical effects and reduced dosing frequency. A problem frequently encountered with conventional sustained release dosage forms is the inability to increase their residence time in stomach and no control over drug delivery, leading to fluctuations in plasma drug level [1, 2]. Envisage of the study was to design of the mucoadhesive gastric retentive formulation of

amoxicillin in order to modified sustained release of the drug in acidic pH region of gastrointestinal tract. Amoxicillin is semi-synthetic amino penicillin with a broad-spectrum bactericidal activity [3-8]. Pectin is nonstarch linear polysaccharides that consist of α -1, 4-galacturonic acid and 1, 2 D-rhamnose with D-galactose and D-arabinose side chains. Pectin is a structural plant polysaccharide, remains an aggregate of macromolecules in acid environments. Pectin aggregates tend to dissociate and digested by a large number of microflora of the colon [9, 10] at neutral-solution pH. Literature and patent survey indicated that calcium pectinate release calcium ion in acidic atmosphere of gastrointestinal tract and converted in to gel form. Carbopol 934P and hydroxypropylmethyl cellulose are important mucoadesive hydrophilic polymers used for blending in pectin in order to change physiochemical nature of calcium pectinate gel bead [11-12].

In the present study, oil entrapped hydrophilic polymer (carbopol or hydroxyl propyl methyl cellulose) blend calcium pectinate buoyant gel bead of amoxicillin was designed, using sodium bicarbonate as gas forming agent. The polymeric blended calcium pectinate enhances mucosal penetration of the drug that may serve as potential vehicle in the targeting of the antibiotics in to infectious microbial lesion present mucosal gel in gastric region. The formulation was continuous maintain minimum inhibition concentration of the drug in the infection site and full fill obstruction of drug amount which could not arrived through blood due to absence of circulatory rout in the mucosal gel. Hence gastric retentive and mucoadesive delivery systems of the present investigation potentially allow increasing penetration of the drug inside mucus gel. Hence, the formulation stabilised appropriate drug concentration around the infection lesion and this will be insure for complete termination of the infection in the acidic region of the gastrointestinal tract. The primary objective of the work was to develop a reliable formulation of amoxicillin that has all the advantages of a floating single unit dosage form but is devoid of disadvantages of single unit dosage forms, namely sticking or .being obstructed in the gastrointestinal tract. The release behaviour of the gel beads capable of floating in gastric fluid was investigated with the aim to achieve a gastroretentive, multiple units, and controlled release formulation of amoxicillin.

MATERIALS AND METHODS

Amoxicillin was obtained as gift sample from Zydus Cadila (Ahmedabad, India), carbopol 934P and hydroxypropyl methylcellulose K4M was obtained as a gift sample from, Ranboxy laboratory Devash, India. Low methoxy pectin with the degree of esterification of 35% and ethyl cellulose were obtained from S.D. Fine Chem. India. Light mineral oil was obtained from the Central Drug House, India. All other ingredients, reagents and solvents were of analytical grade.

Preparation of polymeric blend oil entrapped bead

Oil entrapped polymeric blend gel bead of calcium pectinate was prepared by ionic gelation method. Pectin blended dispersion of hydroxypropyl methylcellulose (HPMC) or carbopol 934P was prepared by succively mixing of aqueous pectin solution (1.5 -2.0 m/v) with 0.5-1.0 % m/v slurry of the hydrophilic polymer with thoroughly stirring with a magnetic stirrer for 15 minutes. An appropriate amount of amoxicillin (0.75 w/v) and calcium carbonate (0.5-1.00 w/w) were dispersed uniformly into 20 ml of polymeric blended pectin mixture with continuous starring until a uniform dispersion was obtained. The mixture was emulsified with 05-15 m/v of light mineral oil using Silveson emulsifier (Hicon, India) maintained continuous stirring with the oil at 500 rpm for 5 min. The resultant drug loaded emulsions was dropped through a 21G syringe needle into 100 mL of 0.45 mol ml⁻¹ of calcium chloride solution, 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 micro gel beads of amoxicillin loaded calcium pectinate blended, either hydroxypropyl methylcellulose (HOB) or carbopol 934P (COB) gel beads took place after 5 minutes of curing time, the formed beads were washed with distilled water and collected by filtration and dried at 40°C for 6 hr.

Coating of gel beads

Formulated gel bead evaluated and selected batch was optimized by coating with ethyl cellulose (EC). The coating parameters were 5-10% (m/V) EC solution in acetone and coating times was fixed (5 - 10 min). Gel beads (2 g) were placed in a fluidized bed dryer (TG 100, Retsch, Germany) and the coating solution was sprayed on the fluidized beads using a spray gun for a period of 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 EC coat on the gel beads (table 2).

Table 1: Composition of drug loaded calcium pectinate gel bead

Formulation no		Drug m/m	Gum Pectin/Polymer m/v	Oil m/v	Calcium carbonate w/w
Pectin/carbopol (COB)	Pectin/HPMC (HOB)				
F ₁	C ₁	0.75	1.5:1.0	05	0.50
F ₂	C ₂	0.75	1.5:1.0	10	0.50
F ₃	C ₃	0.75	1.5:1.0	15	0.50
F ₄	C ₄	0.75	1.5:1.0	05	1.0
F ₅	C ₅	0.75	2.0:0.5	05	0.50
F ₆	C ₆	0.75	2.0:0.5	10	0.50
F ₇	C ₇	0.75	2.0:0.5	15	0.50
F ₈	C ₈	0.75	2.0:0.5	05	1.0

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

Formulation code	EC concentration % m/v	Time of coating (min)	% drug release t _{480(min)}	R
F ₁₄ F	5	74	74	0.9426
F ₂₄ F	5	70	70	0.9323
F ₃₄ F	10	64	64	0.9224
F ₄₄ F	10	59	59	0.9124

Size and Morphology

Particle size of the prepared beads were determined in three set using an optical microscope (Model BH-2, Olympus, Japan) fitted with a stage and an ocular micrometer. 20 dried beads were measured for calculation of mean diameter. The external and internal morphology of micro gel beads were studied by scanning electron microscopy. The micro beads were coated with gold palladium under an argon atmosphere using a gold sputter module in a high vacuum evaporator. The coated samples were then observed with a scanning electron microscope.

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 bottom of the flask were recovered separately. The percentage of floating was measured by visual observation [13].

Determination of drug loading and encapsulation efficiency

Accurately weighed (100mg) grounded powder of beads was soaked in 100 ml phosphate buffer (pH 7.5) and allowed to disintegrating completely for 4 h. The resulting dispersion was sonicated using probe sonicator (UP 400 s, Dr. Hielscher GmbH, Germany) for 30 minutes and then filtered through 0.45 μm filter. The polymeric debris was washed twice with fresh phosphate buffer to extract any adhered drug and the drug content was determined by spectrophotometrically at 334.5 nm against constructed calibration curve. The encapsulation efficiency (EE) was calculated according to the relationship

$$\%EE = \frac{\text{calculated drug content}}{\text{theoretical drug content}} \times 100$$

***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 [14]. An accurately weighed 50 mg amount of the bead (containing 19 – 21 mg) of active drug dropped in 900 ml of both fasted state(simulated gastric fluid ,SGF , pH 1.2) and fed state (phthalate buffer solution, pH 3.4) conditions maintained at a temperature of $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ and stirred at a speed of 50 rpm. At different time intervals, a 10 - mL aliquot of the sample was withdrawn at 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 h and the volume was replaced with an equivalent amount of plain dissolution medium. The collected samples were filtered and suitably diluted and analyzed at λ_{max} 334.5 nm using a UV-visible spectrophotometer (Shimadzu). Drug release data were corrected for the values of the drug loss during sampling and during presence in acidic pH. All the tests were carried out in triplicate. Additionally, an experimental batch BE and BF containing 10 mg amoxicillin 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.

Statistical analysis

The experimental results were expressed as mean \pm SD (standard deviation). Student's t test was applied to determine the level of significance.

RESULTS AND DISCUSSION

Evaluation of blended micro gel bead

Morphology of bead: - Pectin form gel by ionotropic gelation with cross linked with divalent calcium ions. When polymeric blended pectin dispersion was dropped into calcium chloride solutions, spherical gel beads were formed instantaneously. Pectin helped to emulsify the mixture of water and oil phase during the homogenization process and its emulsion stabilization property could be explained by its surface-active ability to reduce the interfacial tension between the oil and water phases. The formulation composition and physico-chemical properties of the various batches of the amoxicillin floating beads were shown in Tables 1 & 3 respectively. Scanning electron micrographs (SEM) of amoxicillin-loaded oil entrapped blended with either HPMC (formulation HOF) or carbopol 934P (formulation COF) batches are shown in Figures 1(a) and (b), respectively. Gel bead of calcium pectinate blended with carbopol 934P batch F₄ was white, translucent and rigid. The surface of this batch was appeared smooth and the presence

of minor projections attributed to the presence of insoluble drug particles in the bead matrix, which was in contrast to C₄ batch with a rough surface (Fig. 1b).

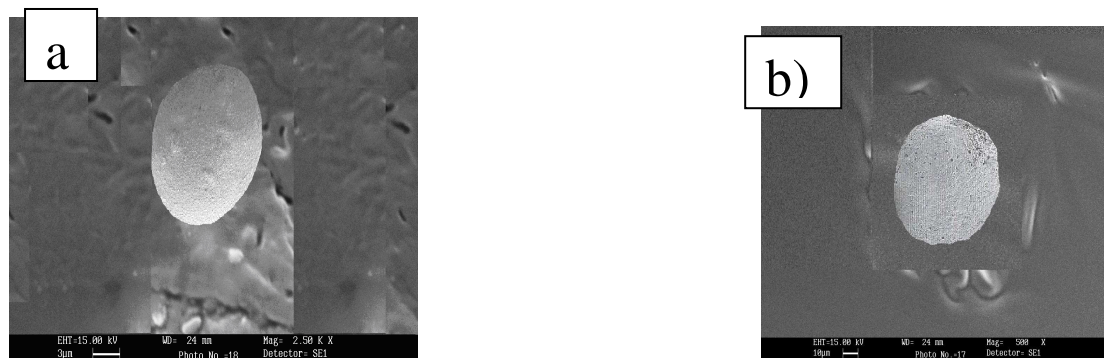


Fig. 1 Outer structure of dried oil entrapped blended polymeric bead of a) batch F4 and b) batch C5 under scanning electron microscope

Floating ability: - The floating ability was evaluated in phthalate buffer solution (pH 3.4). The percentages floated on surface of the medium were evaluated and are shown in Table 3. Gel microbeads were produced due to gelation and cross linking by Ca⁺⁺ ions provided a gel barrier at the surface of the formulation. The calcium carbonate was produced effervesced and released carbon dioxide and calcium ions. The released carbon dioxide was entrapped in the gel network, which was invariably a three dimensional network resulted to produce a buoyant formulation. The floating ability of the formulation mainly depends on calcium carbonate and blended polymeric concentration. The beads containing 1.0% of the gas-forming agent (calcium carbonate) demonstrated good floating ability 96±1.9 (batch F₄) and 88 ± 1.8 (batch C₄). 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 consequently the amount of CO₂, evolved are responsible to increase floating ability of beads. The factors contributing for floating appeared to be the porous structure of beads, low relative densities of mineral oil (0.84 g mL⁻¹) as compared to that of gastric media (1.004 g mL⁻¹), facilitation of air entrapment by the oils.

Table 3: Characterization of prepared formulation of calcium pectinate gel beads

Formulation no		Diameter (mm) ^{abc}		Floating ability(%) ^{a,c}		Encapsulation Efficiency (%w/w) ^{a,c}		Drug content % ^{a,d,c}	
COF)*	(HOF)**	COF	HOF	COF	HOF	COF	HOF	COF	HOF
F ₁	C ₁	0.81±0.2	0.92±0.4	89±1.4	80±1.6	88±0.3	78±0.8	54±1.24	46±2.15
F ₂	C ₂	0.89±0.4	0.97±0.6	86±1.3	77±1.8	81±0.6	75±0.4	48±1.31	38±1.41
F ₃	C ₃	0.95±0.4	1.24±0.8	80±1.5	72±1.3	75±0.9	77±0.3	46±1.25	36±1.31
F ₄	C ₄	0.86±0.4	0.95±0.9	96±1.9	88±1.8	94±0.7	90±0.5	58±1.22	57±1.32
F ₅	C ₅	1.0±0.7	1.15±0.6	78±1.6	72±1.2	90±0.3	86±0.9	50±1.46	44±1.14
F ₆	C ₆	1.16±0.3	1.31±0.8	70±1.4	66±1.3	86±0.1	84±0.8	48±1.25	42±1.31
F ₇	C ₇	1.28±0.8	1.49±0.3	65±1.4	58±1.8	89±0.4	87±0.5	51±1.36	45±1.33
F ₈	C ₈	1.39±0.3	1.38±0.9	73±1.9	66±1.4	87±0.2	85±0.7	49±1.23	43±1.34

COF* = Pectin/carbopol; HOF** = Pectin/HPMC; a. = Mean ± SD; b n = 20; c n = 3; d Drug content in each 100 mg of bead.

Encapsulation efficiency and drug loading: - The effects of various formulation parameters on the encapsulation efficiency of floating beads are shown in Table 3. F₄ batch of COF

formulation was showed highest amoxicillin loading ($58 \pm 1.22\%$ w/w) and batch F_3 showed lowest drug loading (46 ± 1.25 w/w) and HOF formulation of batch C_4 was showed highest drug loading (57 ± 1.23) and batch C_3 showed lowest drug loading ($36 \pm 1.31\%$). Encapsulation was found to be consistently higher in all the batches of formulation COF (ranges as: $75 \pm 0.9 - 94 \pm 0.7\%$) and formulation of HOF (ranges as: $75 \pm 0.4 - 90 \pm 0.5\%$). Encapsulation efficiency was decreased as the oil concentration was increased. No significant ($P > 0.05$) effect was observed for calcium carbonate and calcium chloride on encapsulation efficiency of bead.

In vitro drug release: - *In vitro* drug release study of amoxicillin gel beads was carried out in the fasted state (SGF solution of pH 1.2), and in the fed state (phthalate buffer of pH 3.4) for a period of 8 h. Gel beads were exhibited a biphasic amoxicillin release profile as an initial rapid drug release phase (burst effect) was followed by a slower, gradually declining drug release phase was extended up to 8 h (Fig. 2). Release of the drug from batches F_4E and batch C_4F were $57.4 \pm 2.0\%$ and $68.0 \pm 1.5\%$ in empty state condition and fed state respectively. While the drug was released within 8 h from the batches C_4E and C_4F were $47 \pm 1.2\%$ and $46 \pm \%$ respectively. Experimental reference capsule was filled with 10 mg of amoxicillin and study, *in vitro* drug release was observed $62 \pm 1.8\%$ (batch BE) and $72.9 \pm \%$ (batch BF) respective to empty and faded condition within 2 h and could not sustain the release of the drug over 8 h, but rather exhibited a rapid first-order decline. This release behaviour substantiates the use of amoxicillin emulsion gel beads as a drug delivery system for modifying the release characteristics of the drug. The two-way analysis of variance (ANOVA) revealed a significant difference between *in vitro* drug release profiles of amoxicillin in the fed and fasted states at a 95% confidence interval ($p < 0.05$). Various release kinetic models were applied to elucidate the mechanism of drug release from the floating gel beads in the fed state. Drug release from the optimized formulations F_4 followed the Higuchi ($R = 0.924$, $n = 0.36$) and Peppas models ($R = 0.912$, $n = 0.36$), respectively, suggested a diffusion based mechanism of the drug release as the diffusion exponent values were less than 0.45.

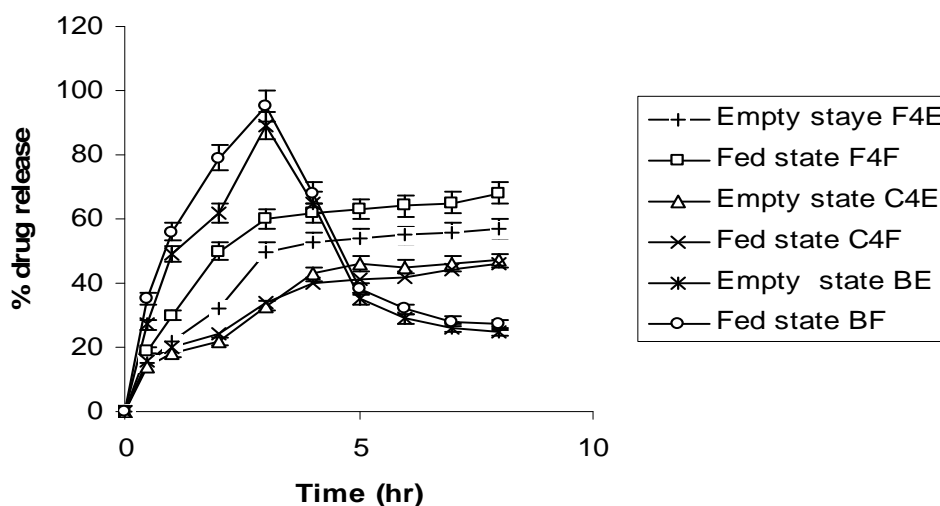


Fig. 2 Comparative drug release profile from different batches at fasted and fed state condition

Behaviour of COF gel beads formulation were exhibited a faster release of the drug while HOF formulation gel beads were showed a relatively slow release this was attributed due to higher viscosity of hydroxypropyl methylcellulose solution than carbopol and to greater partitioning of the drug to carbopol compared to hydroxypropyl methylcellulose. Nonenteric polymer: ethyl cellulose (EC) was selected for coating of gel beads due to its stability in gastric pH and based on the reports for the use of EC for coating on floating micro particles to modify the drug release

[15]. Batch F₄ which exhibited the highest diffusion exponent in fed state conditions, selected for coating. The desirable dependent response was showed zero-order release pattern from EC-coated formulation in the fed state. The release of amoxicillin from formulation F₁₄F gel beads was highest (78± 1.7%) whereas formulation F₃C₄ exhibited the lowest drug release of 62.3 ± 2.0% at the end of 8 h (Fig. 3). It is generally known that the mode of the drug release from gel beads coated with a water insoluble membrane/polymer (reservoir type), was penetration of liquid, dissolution of the drug to form a saturated solution (as long as undissolved drug is present) and partitioning of drug into the polymeric membrane, resulting in drug diffusion through the membrane [16]. A similar mechanism can be suggested for the release of amoxicillin from the floating gel beads. Dissolution independent parameters for coated beads were tabulated in Table 2. The formulation batch F₁₄F exhibited maximum dissolution efficiency (74.4 %) after 480 min and dissolution profile that best fitted zero-order release and *R* value of 0.9426 was optimized batch of the controlled-release formulation of amoxicillin.

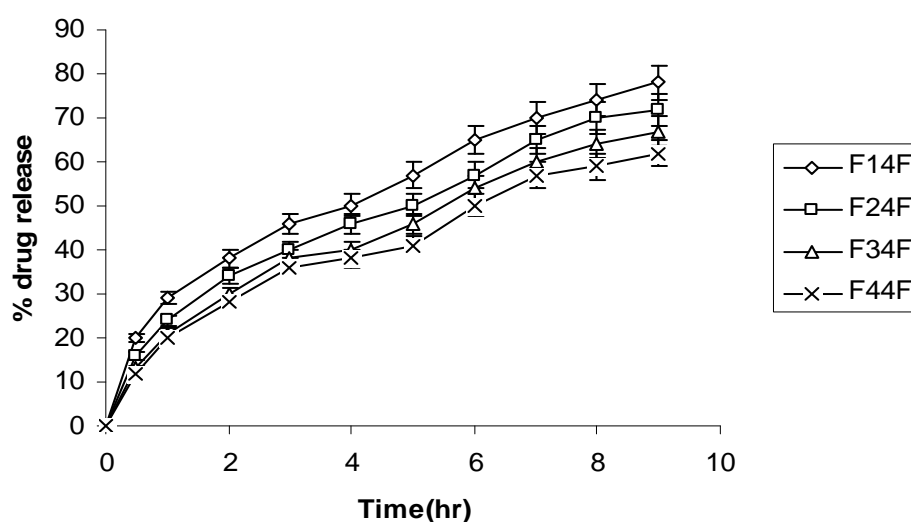


Fig. 3 Comparative drug release profiles of EC-coated gel beads

CONCLUSION

The optimized gel bead of amoxicillin was an excellent buoyant formulation in order to targeted controlled release of the drug in gastric region. Presences of mucoadhesive polymer in the formulation help to penetrate amoxicillin in side mucosal gel. Hence the formulation helps to release the drug at surface or very close to infectious lesion. The gel beads have promising potential for the delivery of amoxicillin at stomach site and may be very useful for targeting the drug at the site of infection and also for *H pylori* eradication.

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