

DEVELOPMENT OF MULTIPARTICULATE-FLOATING SYSTEM FOR PULSATILE RELEASE OF MONTELUKAST SODIUM

Ramya Krishna T^{1*}, Suresh B¹, Prabhakar Reddy V¹, Pavani V¹, Nalini Krishna Reddy²

¹ St. Peter's Institute of Pharmaceutical Sciences, Hanamkonda, Warangal, A.P, India

² Talla Padmavathi Pharmacy College, Orus, Warangal, A.P, India

Corresponding Author Email: ramyatalakoti@gmail.com

ABSTRACT

The purpose of this work was to develop a multi-unit alginate beads for floating- pulsatile release of montelukast sodium intended for chronopharmacotherapy. Sodium alginate has been investigated as a carrier for an intragastric floating drug delivery by means of calcium alginate beads. Floating pulsatile concept was applied to increase the gastric residence time of the dosage form having lag phase followed by burst release. Floating alginate beads were prepared by ionotropic gelation method with calcium carbonate being used as gas forming agent. The alginate solution was dispersed with carbonate salt and then extruded into acidified solution of calcium chloride. Acidity of gelation medium increased the pores in the structure of beads. This is due to carbon dioxide generated from reaction of carbonate salt with acid. The obtained beads were porous with bulk density <1 and $F_{150\%}$ of 12-24hrs. The beads showed two phase release pattern with initial lag time during floating in acidic medium followed by rapid pulse release in phosphate buffer. This approach can be used for variety of drugs suitable for chronopharmacotherapy. The surface morphology of beads was determined by scanning electron microscopy (SEM). The excipients used in this study did not alter physicochemical properties of the drug, as tested by the Fourier transform infrared spectroscopy (FTIR) and differential scanning calorimeter (DSC).

KEYWORDS

Floating-pulsatile drug delivery, calcium alginate beads, montelukast sodium, chronopharmacotherapy.

INTRODUCTION

Gastroretentive drug delivery system prolongs gastric residence time, thereby targeting site-specific drug release in upper gastrointestinal tract (GIT). Floating drug delivery system is the widely used technique for gastroretention¹. Biodegradable, natural polysaccharides like pectin, guar gum, chitosan, sodium alginate and gellan gum have been used in controlled drug delivery²⁻⁶. Alginate is a bioadhesive, biodegradable polysaccharide which contains varying amounts of 1, 4'- linked β -D-mannuronic acid, α -L-guluronic acid residues. It forms a bioadhesive and stable gel with divalent cations such as Ba^{2+} , Sr^{2+} and Ca^{2+} which enabled widespread use for sustained release of drugs^{7, 8}. They can also be function as carriers as bifidobacteria⁹ and used for pulsatile

release of drugs since alginate beads are stable in acidic media and easily depreated in alkaline media. Alginate beads obtained by ionotropic crosslinking of these polymers have been used to develop floating drug delivery¹⁰. Various approaches like use of volatile oils, freeze drying, and entrapment of gas or gas forming agent have been used to induce buoyancy in cross-linked beads. The oil containing beads have limitations of volatilization or leaching of oil, coalescence of oil droplets yields beads of wider particle size distribution. Hence, beads formed by incorporating gas forming agent ($CaCO_3$) are simple to produce,¹¹⁻¹³ which have been attempted. The floating property for beads is induced by evolution of carbon dioxide when in contact with acidic environment followed by the ability of polymer gel

to entrap it which decreases their density below one. Violent gas generation, burst release, alkaline environment, disintegration of dosage form are limitations of these dosage form.

The principle involved in pulsatile drug delivery system is rapid of a certain amount of drug within short time period after a predetermined off-release period, lag time ¹⁴. This drug delivery has been used for various diseases like

(i) Chronopharmacotherapy of diseases which show circadian rhythms in their pathophysiology ¹⁵; (ii) Avoiding degradation of active ingredients in upper GI tract, e.g. proteins and peptides ¹⁶; (iii) For time programmed administration of hormones and many drugs such as isosorbide dinitrate, respectively to avoid suppression of normal secretion of hormones in body that can be hampered by constant release of hormone from administered dosage form and development of resistance ¹⁷⁻²²; (iv) To avoid pharmacokinetic drug-drug interactions between concomitantly administered drugs ²³, etc. Montelukast sodium, [R-(E)]-1-[[[1-[3-[2-(7-chloro-2-quinolinyl) ethenyl] phenyl]-3-[2-(1 hydroxymethylethyl) phenyl] propyl] thio] methyl] cyclopropaneacetic acid, monosodium salt, an acid insoluble selective cysLT1 leukotriene receptor antagonist indicated for the prophylaxis and chronic treatment of asthma ²⁴. The objective of the present study was to develop a multiparticulate, floatin-pulsatile drug delivery system of alginate by a process of evolution of CO₂ during crosslinking in acidic medium for obtaining no drug release during floating time followed by pulse drug release in

small intestine. The obtained beads were evaluated for drug content, size analysis, *in vitro* floating properties and *in vitro* drug release.

EXPERIMENTAL

Materials

Montelukast sodium was obtained as gift sample from Dr. Reddy's Laboratories, Hyderabad, India. Sodium alginate was purchased from S.D Fine Chem. Ltd, Mumbai. All other reagents and chemicals used were analytical grade.

Methods

Preparation of Beads

The alginate beads were prepared by the ionotropic gelation technique. A solution was prepared by dissolving 100mg montelukast sodium in 5mL of distilled water. Sodium alginate solution (3 and 4 % w/v) was prepared by dissolving sodium alginate in 30mL of distilled water. Then, varied amounts of calcium carbonate (gas forming agent) was added and uniformly mixed, as shown in **Table I** ¹¹. Then, the resultant dispersion was sonicated for 30min to remove any air bubbles and was dropped via a 16-gauge syringe needle into 80mL of 2% w/v calcium chloride solution containing 10% acetic acid. Then the solution containing suspended beads was stirred at 100rpm using magnetic stirrer for 15min to improve the mechanical strength of the beads and also to prevent aggregation of the formed beads. The fully formed beads were collected, washed with distilled water and subsequently oven dried at 50°C for 4h ¹⁰.

Table I. Formulation composition of Montelukast sodium floating alginate beads

CODE	Montelukast Sodium (mg)	CaCO ₃ (mg)	Sodium Alginate (mg)	CaCl ₂ solution 2%w/v (ml)	Acetic Acid (10%v/v) (ml)
A1	100	75	900	72	8
A2	100	150	900	72	8
A3	100	225	900	72	8
A4	100	300	900	72	8
A5	100	450	900	72	8
A6	100	600	900	72	8
A7	100	75	1200	72	8
A8	100	150	1200	72	8
A9	100	225	1200	72	8
A10	100	300	1200	72	8
A11	100	450	1200	72	8
A12	100	600	1200	72	8

Particle Size Analysis

The particle size (n=20) of the calcium alginate beads was measured with a 12cm vernier calipers and their average diameter was recorded^{25, 26}.

Scanning Electron Microscopy

The shape and surface morphological examination of the surface structure of dried beads were carried out by scanning electron microscopy (Cambridge Stereoscan S120, Cambridge, UK) operated at an acceleration voltage of 5 Kv¹.

Buoyancy Test

The prepared beads were studied for buoyancy²⁷ and floating time using USP 23 type II dissolution apparatus, one hundred beads of each batch were placed in 900mL of 0.1N HCl (pH 1.2) containing 0.02% v/v Tween 80 and agitated at 100rpm, temperature was maintained at 37±2°C. Number of sinking beads was observed visually¹⁰.

Entrapment Efficiency and Drug Loading

The entrapment efficiency of beads was determined by the reported method¹⁰. 100mg beads were dissolved in 100ml phosphate buffer (pH 7.4) by shaking on rotary shaker (Steelmet Industries, Pune, India) at 200 rpm for 24h. Then the resultant dispersion was filtered through a 0.45

µm filter and analyzed at 243nm using UV spectrophotometer.

The encapsulation efficiency was determined by equation

$$\text{Encapsulation efficiency (\%)} = \text{AQ} / \text{TQ} \times 100;$$

Where AQ is the actual drug content of beads and TQ is the theoretical quantity of drug present in beads¹⁰.

The drug loading was calculated according to the following equation

$$\text{Drug Loading (\%)} = \text{WD} / \text{WT} \times 100$$

DL: drug loading; WD: the weight of the drug loaded in the microspheres; WT: the total weight of the microspheres²⁸.

Differential Scanning Calorimetry

Thermograms of montelukast sodium, placebo beads and drug loaded beads were obtained by using Mettler-Toledo DSC 821e instrument (Mettler Toledo, Greifensee, Switzerland) equipped with an intracooler. Indium standard was used to calibrate the DSC temperature and enthalpy scale. 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⁻¹²⁹.

Infrared Spectroscopy

FTIR analysis measurements of montelukast sodium, placebo beads and drug loaded beads were obtained JASCO V5300 FT-IR (Tokyo, Japan). The pellets were prepared on KBr-press (Spectra Lab, Pune, India) under hydraulic pressure of 150 kg/cm²²⁹.

In Vitro Drug Release Studies

In vitro drug release studies of the beads were carried out by using USP 23 type I dissolution test apparatus (Electrolab TDT-06P, Mumbai, India). A weighed amount of beads equivalent to 10mg of montelukast sodium was placed in the dissolution basket and the drug release study was carried out in 0.1N HCl containing 0.5% SLS for initial 6h, followed by dissolution in phosphate buffer, pH 7.4 containing 0.5% SLS, each 900mL, maintained at 37±2°C and agitated at 100rpm (n=3). Periodically samples were withdrawn and filtered through Whatman filter paper 41 and concentration of montelukast sodium was measured spectrophotometrically at 243nm¹⁰.

RESULTS AND DISCUSSION

Preparation of Beads

Calcium alginate beads is an approach of multiparticulate pulsatile drug delivery system for drugs and macromolecules because of its high pH dependent characteristics of swelling and drug release^{14,30}. The calcium alginate beads containing CaCO₃ were produced instantaneously by ionotropic gelation in which intermolecular cross-links were formed between the divalent calcium ions and negatively charged carboxyl groups of the alginate molecules³¹. The calcium is ionically substituted at the carboxyl site of alginate strands in presence of solid gel³². The divalent calcium cations fits into electronegative cavities like eggs in an egg-box; from this similitude arises the term "Egg Box" model.

The cross-linking sites that occur when a polyvalent cations cause interpolysaccharide binding are called junction zones³³.

Sodium alginate contains anionic groups i.e. carboxyl and hydroxyl groups in its structure, which exhibit the property of electrostatic interaction. When sodium alginate comes in contact with divalent metal ions (Ca²⁺ ions), the ionic interaction occurs between the Ca²⁺ ions and carboxyl groups present on alginate chain. The divalent cation i.e. Ca²⁺ competes with the Na⁺ ions for the anionic sites and replaces it, thus bringing the two polymer chains together. Ca²⁺ ions get accommodated in the interstices of two polyuronate chains having a close ion-pair interaction with carboxylate anion and sufficient coordination by other electronegative oxygen atoms³⁴. This forms an 'egg-box' type arrangement³⁵. In addition to ionic interaction, there occurs hydrogen bonding between of two polysaccharide chains. In stomach alginate is not digested by gastric enzymes and has minimum swelling but undergoes rapid gel relaxation³⁶⁻³⁸.

The beads containing 3% w/v of sodium alginate were spherical in shape and had good mechanical strength. The beads containing 4% w/v of sodium alginate showed tail formation during preparation. Increase in amount of CaCO₃ in the beads leads to porous and hollow structure due to liberation of gas. Using 3% w/v of calcium carbonate, the beads formed showed rough surface and irregular shape because of excess liberation of gas. During the formation of calcium alginate beads, the carbonate salts react with acetic acid to produce the calcium alginate structure leaving gas bubbles or pores resulting in the highly porous structure.

Particle Size Analysis

The formed alginate beads were almost spherical. The particle sizes of beads were shown in Table II. The mean particle sizes of beads were between 0.45 ± 0.003mm and 0.77 ± 0.007mm. Different weight ratios of CaCO₃ to alginate were used to determine the effect of the gas forming process on

the size of the beads. As the concentration of the sodium alginate increases during the beads preparation, the shape of the beads became almost spherical and the mean bead diameter increases due to increase in micro-viscosity of the polymeric dispersion with increase in concentration of alginate^{39, 40}. The increase in

diameter with incorporation of CaCO_3 was due to the reason that when CaCO_3 reacted with acetic acid present in gelation (cross-linking) medium, CO_2 formed and escaped from the bead matrix. This has produced a porous high volume bead with increased diameter⁴¹.

Table II. Physico-chemical evaluation parameters of floating alginate beads

Formula code	Floating property	Duration of Floatation (hrs)	Mean diameter (mm) (n=20)	Drug Loading (%)	Entrapment Efficiency (%)
A1	--	-	0.45±0.003	6.71	81.00
A2	+-	24	0.49±0.002	7.36	81.42
A3	++	24	0.58±0.004	7.55	81.50
A4	++	24	0.63±0.005	7.77	82.56
A5	++	24	0.67±0.005	7.82	83.60
A6	++	24	0.73±0.007	9.60	84.00
A7	--	-	0.52±0.009	6.36	80.84
A8	+-	24	0.55±0.001	6.42	81.27
A9	++	24	0.60±0.001	7.60	81.42
A10	++	24	0.65±0.002	7.92	81.84
A11	++	24	0.67±0.003	8.11	83.16
A12	++	24	0.77±0.003	9.67	84.60

-- = completely sink, +- = partial floating, ++ = complete floating

Scanning Electron Microscopy

The surface and cross sectional SEM pictures for optimized formulation of floating beads were shown in **Figure 1A and B**. Incorporation of Ca^{2+}

ions might have contributed to the homogenous alginate bead formation. In fact, CaCO_3 has been reported to be used as a gelling agent to aid the internal gelation of the alginate^{41, 42}.

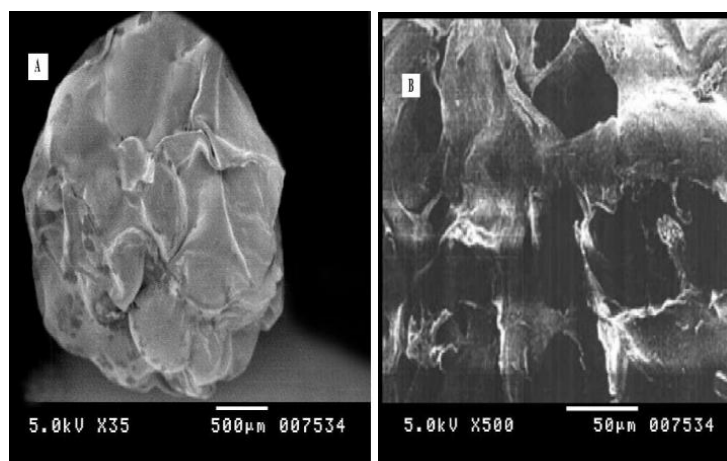


Figure 1. Scanning electron microscopy photomicrographs of A6 formulation
A) Cross-section, B) surface morphology

Buoyancy Test

The buoyancy of beads is directly related to performance of floating pulsatile drug delivery system since lag time for beads is equivalent to their floating time¹. The floating ability of the beads was studied by determining buoyancy and time required for sinking all the beads under study. Surface tension of gastric juice was stimulated by the use of surfactant²⁷. The floating property of the beads was shown in **Table II**. Alginate solution upon contact with acidic medium, gelation and crosslinking by Ca^{2+} ions occurred to provide a gel barrier at the surface of the formulation. The calcium carbonate effervesced, releasing carbondioxide and calcium ions. The carbondioxide produced was entrapped in the gel network producing buoyant formulation and then the calcium ion reacted with alginate producing a cross-linked three dimensional gel network that restricted further diffusion of carbondioxide and drug molecules and resulted in an extended period of floating and drug release⁴³⁻⁴⁵. The floating property of the beads may be attributed to low apparent density and the porosity of the beads. Beads containing 0.25 and 0.5% of CaCO_3 were completely sinked and partially floated. The beads containing 0.75-2% of the gas forming agent demonstrated good floating ability. Increase in

calcium carbonate concentration, increased the floating property of beads¹⁰⁻¹³.

Entrapment Efficiency and Drug Loading

The entrapment efficiency and drug loading of beads were found to be in range of 81.00 to 84.6% and 6.71-9.67%. The encapsulation efficiency increased with increase in amount of calcium carbonate. Effect of calcium carbonate can be attributed to the formation of alkaline microenvironment inside the bead enhancing drug solubility combined with the effervescent action-giving rise to modifications of bead matrix in situ. Collective action exerted by the increased amount of calcium carbonate leads to the formation of prominent porous structures due to entrapment of generated gas. This entrapment leads to the coalescence of gas bubbles, which pushed the internal matrix towards periphery forming thick boundaries minimizing drug leaching¹⁰. The entrapment efficiency and drug loading of beads were shown in **Table II**.

Differential Scanning Calorimetry

DSC thermograms of montelukast sodium, placebo beads and drug loaded beads are depicted in Figure 2. Montelukast sodium showed sharp melting endotherm at 56.1°C and placebo beads showed melting endotherm at 187°C and drug loaded beads showed melting endotherm at 69.4°C and 189.8°C.

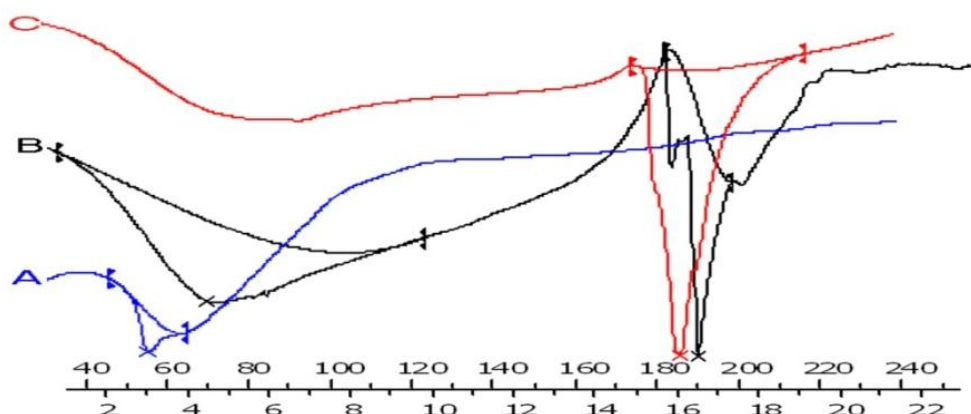


Figure 2. Differential Scanning calorimetry (DSC) thermograms of A) Montelukast Sodium B) Drug loaded beads C) placebo beads

Infrared spectroscopy

FTIR spectra of montelukast sodium, placebo beads and drug loaded beads were shown in **Figure 3**. In case of montelukast sodium, the bands were observed in the region of 3000-3700 cm^{-1} (i.e. 3394, 3554, 3612 and 3667 cm^{-1}) due to -OH stretching. Bands was observed at 2949 cm^{-1} due to C-H stretching, at 1739 cm^{-1} due to C=O stretching and at 1551 cm^{-1} , 1505 cm^{-1} due to N-H bending respectively.

In case of sodium alginate spectrum, the bands around 1024 cm^{-1} due to C-O-C stretching are

attributed to its saccharide structure. In addition, the bands at 1593 cm^{-1} and 1417 cm^{-1} are assigned to asymmetric and symmetric stretching peaks of carboxylate salt groups ⁴⁶. The alginate fraction have shown bands in the region 950–815 cm^{-1} (i.e. 945 cm^{-1} , 912 cm^{-1} and 878 cm^{-1}) which were due to polyguluronic acid and polymannuronic acid sequences in the alginate backbone ⁴⁷.

The characteristic peaks of montelukast sodium were not altered after encapsulation indicating no chemical interaction between drug and polymer.

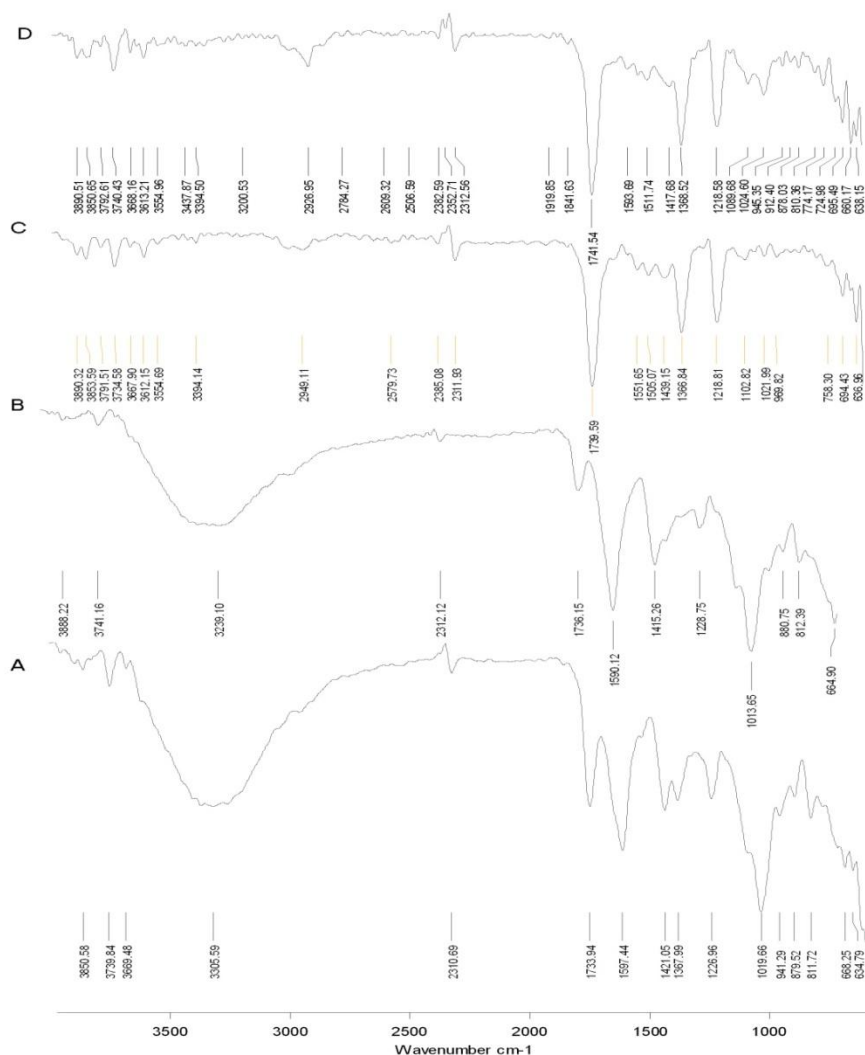


Figure 3. Fourier transform infrared spectra (FTIR) of
A) Drug loaded beads B) Placebo beads C) Sodium alginate D) Montelukast sodium

In vitro drug release studies

In vitro release profiles obtained from various formulations have been shown in Figure 4a and b. Release studies in 0.1N HCl (pH 1.2) for 6hrs showed 4.9–7.9% and no release of the drug in acidic medium irrespective of time for 2 and 4% w/v alginate beads, respectively. After this lag, it was followed by pulse with complete drug release within 30–45 min in phosphate buffer, pH 7.4. The porous beads also showed excellent lag in drug release at acidic pH that may be due to insolubility of drug and alginate. At acidic pH, calcium alginate

may get protonated into insoluble form having reduced swelling. The second phase of pulsed release in phosphate buffer, pH 7.4, can be attributed to rapid swelling and gel relaxation of calcium alginate gel at alkaline pH (10). Secondly at pH >7 montelukast sodium is freely soluble that resulted in rapid and complete drug release. Beads containing 4% w/v alginate showed no release in acidic medium and it was followed by pulse release in alkaline medium but when compared to 2% w/v alginate beads there was slow drug release in alkaline medium.

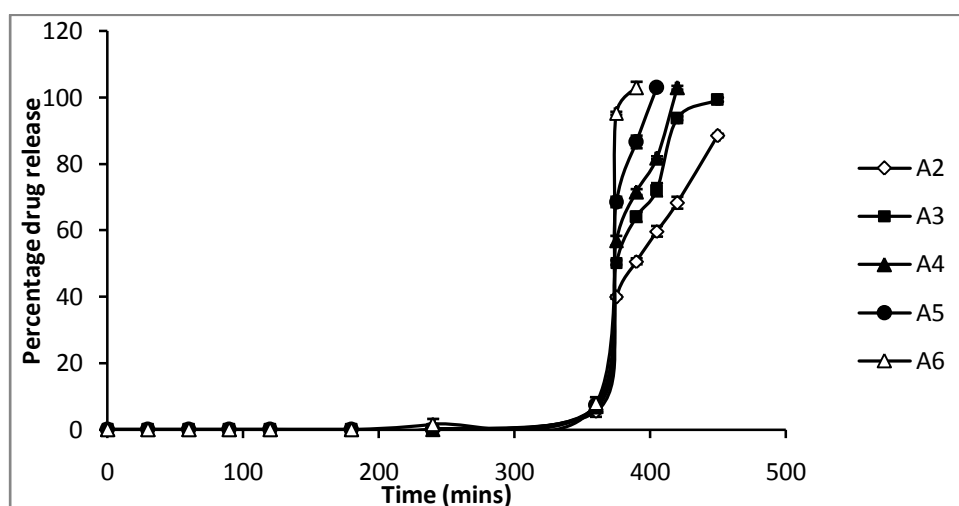


Figure 4a. Cumulative percentage of drug release of 3% sodium alginate beads

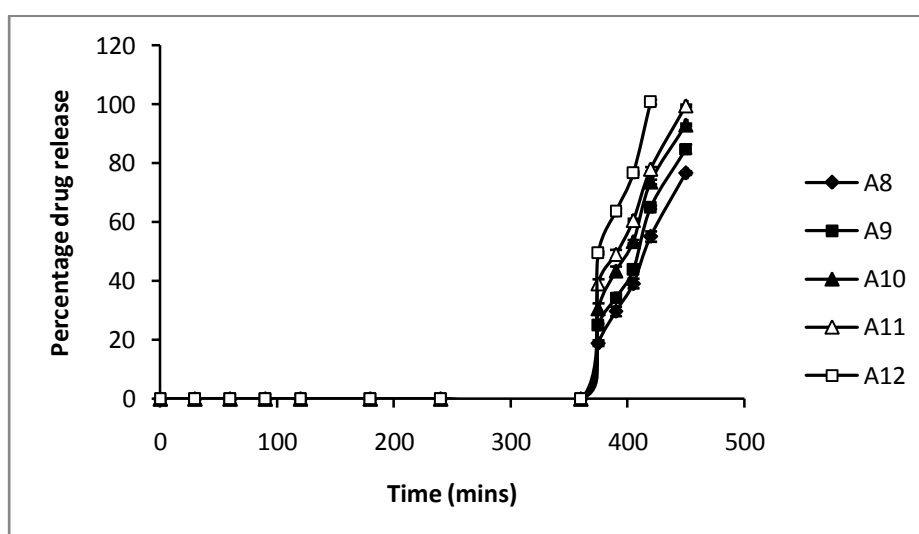


Figure 4b. Cumulative percentage of drug release of 4% sodium alginate beads

CONCLUSION

Multiple-unit floating-pulsatile beads of montelukast sodium were prepared to provide chronotherapy. The formulation A12 exhibited optimum release of montelukast sodium with excellent floating properties. Developed formulations showed instantaneous floating with very less drug release in acidic medium followed by a pulse release in alkaline medium. Drug release from beads in alkaline environment was influenced by sodium alginate concentration. Overall, the buoyant beads provided a lag phase in acidic medium while showing gastroretention followed by a pulsatile drug release that would be beneficial for chronotherapy of asthma. This work can be extended for variety of drugs suitable for chronotherapy.

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REFERENCES

1. S. Sameer and P. Atmaram, Low density multiparticulate system for pulsatile release of meloxicam, *Int. J. Pharm.* 313 (2006) 150-158.
2. K. S. Soppimth, A. R. Kulkarni and T. M. Aminabhavi, Controlled release of antihypertensive drug from the interpenetrating network poly (vinyl) alcohol, guar gum hydrogel microspheres, *J. Biomater. Sci. Polym. Ed.* 11 (2000) 27-43.
3. J. Hwagno, G. W. Skinner, W. W. Harcu and P. E. Barnum, Pharmaceutical application of naturally occurring water soluble polymer, *Pharm. Sci. Technol. Today* 1 (1998) 254-261.
4. Z. Aydin and J. Akbuga, Preparation and evaluation of pectin beads, *Int. J. Pharm.* 137 (1996) 133-136.
5. K. Kedziereuciz and C. Lemory, Effect of the formulation on the in vitro release of propranolol from gellan beads, *Int. J. Pharm.* 178 (1999) 129-136.
6. L. Whithead, J. H. Collete and J. T. Fell, Amoxicillin release from a floating dosage form based on alginates, *Int. J. Pharm.* 21 (2000) 45-49.
7. G. T. Grant, E. R. Morris, D. A. Rees, P. J. C. Smith and D. Thom, Biological interactions between polysaccharides and divalent cations: the egg-box model, *FEBS Lett.* 2 (1973) 195-198.
8. O. Smidsrod and A. Haug, The effect of divalent metals on the properties of alginate solutions. I. Calcium ions, *Acta Chem. Scand.* 19 (1965) 329-340.
9. K. Sultana, G. Godward, N. Reynolds, R. Arumugaswamy, P. Peiris and K. Kailasapathy, Encapsulation of probiotic bacteria with alginate-starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt, *Int. J. Food Microbiol.* 62 (2000) 47-55.
10. S. S. Badve, S. Praveen, K. Aruna and P. P. Atmaram, Development of hollow/porous calcium pectinate beads for floating-pulsatile drug delivery, *Eur. J. Pharm. Biopharm.* 65 (2007) 85-93.
11. B. Y. Choi, H. J. Park, S. J. Hwang and J. B. Park, Preparation of alginate beads for floating drug delivery: effects of CO₂ gas forming agents, *Int. J. Pharm.* 239 (2002) 81-91.
12. S. Parauvathanahalli, Rajinikanth and M. Brahmeshwar, Preparation and *in vitro* characterization of gellan based floating beads of acetohydroxamic acid for eradication of *H. pylori*, *Acta Pharm.* 57 (2007) 413-427.
13. Shishu, G. Neeta and A. Nidhi, Stomach-Specific Drug Delivery of 5-Fluorouracil Using Floating Alginate Beads, *AAPS PharmSci Tech.* 8(2) (2007) E1-E7.
14. A. Kikuchi and T. Okano, Pulsatile drug release control using hydrogels, *Adv. Drug Del. Rev.* 54 (2002) 53-77.
15. T. Sawada, H. Kondo, H. Nakashima, K. Sako and M. Hayashi, Time-release compression-coated core tablet containing nifedipine for chronopharmacotherapy, *Int. J. Pharm.* 280 (2004) 103-111.
16. A. Rubinstein, B. Tirosh, M. Baluom, T. Nassar, A. David, R. Radaï, I. Gliko-Kabir and M. Friedman, The rationale for peptide drug delivery to the colon and the potential of polymeric carriers as effective tools, *J. Control. Release.* 46 (1997) 59-73.
17. J. O. Parker, H.L. Fung, D. Rugginello and J. A. Stone, Tolerance to isosorbide dinitrate: rate of development and reversal, *Circulation* 68 (1983) 1074-1080.
18. P. D. Goldenheim, E. A. Conrad and L. K. Schein, Treatment of asthma by a controlled release theophylline tablet formulation: a review of the North American experience with nocturnal dosing, *Chronobiol. Int.* 4 (1987) 397-408.
19. J. T. Flaherty, Nitrate tolerance: a review of the evidence, *Drugs* 37 (1989) 523-550.

20. A. G. Jimoh, D. L. Wise, J. D. Gresser, and D. J. Trantolo, Pulsed FSH release from an implantable capsule system, *J. Control. Release.* 34 (1995) 87–95.
21. A. Charloux, C. Gronfier, E. Lonsdorfer-Wolf, F. Piquard and G. Brandenberger, Aldosterone release during the sleep-wake cycle in humans. *Am. J. Physiol.* 276 (1999) E43–E49.
22. E. Terasawa, K. L. Keen, K. Mogi and P. Claude, Pulsatile release of luteinizing hormone-release (LHRH) in cultured LHRH neurons derived from the embryonic olfactory placode of the rhesus monkey, *Endocrinology.* 140 (1999) 1432–1441.
23. T. Sawada, K. Sako, K. Yoshihara, K. Nakamura, S. Yokohama and Hayashi, Timed-release formulation to avoid drug–drug interaction between diltiazem and midazolam, *J. Pharm. Sci.* 92 (2003)790–797.
24. N. Kanakadurga Devi, A. Prameela Rani and B. Sai Mrudula, Formulation and evaluation of oral disintegrating tablets of montelukast sodium: effect of functionality of superdisintegrants, *Journal of Pharmacy Research* 3(4) (2010) 803-808.
25. Y. Murata, N. Sasaki, E. Miyamoto and S. Kawashima, Use of floating alginate gel beads for stomach-specific drug delivery, *Eur. J. Pharm. Biopharm.* 50(2) (2002) 221-226.
26. B. Singh, V. Sharma and D. Chauhan, Gastroretentive floating sterculia–alginate beads for use in antiulcer drug delivery, *Chem. Eng. Res. Des.* 448 (2010)1-16.
27. I. El-Gibaly, Development and *in vitro* evaluation of novel floating chitosan microcapsules for oral use: comparison with non floating chitosan microspheres, *Int. J. Pharm.* 249 (2002) 7–21.
28. M. Ninan, Lu Xu, W. Qifang, Z. Xiangrong, Z. Wenji, L. Yang, J. Lingyu and L. Sanming. Development and evaluation of new sustained-release floating microspheres. *Int. J. Pharm.* 358 (2008) 82–90.
29. Y. L. Patel, S. Praveen and P. W. Atmaram, The Effect Of Drug Concentration And Curing Time On Processing And Properties Of Calcium Alginate Beads Containing Metronidazole By Response Surface Methodology, *AAPS PharmSciTech* 7(4) (2006) E1-E7.
30. V. Pillay and R. Fassihi, *In vitro* release modulation from crosslinked pellets for site-specific drug delivery to the gastrointestinal tract I. Comparison of pH responsive drug release and associated kinetics, *J. Control. Release.* 59 (1999) 229–242.
31. P. Sriamornsak and J. Nunthanid, Calcium pectinate gel beads for controlled release drug delivery: I. Preparation and in-vitro release studies, *Int. J. Pharm.* 160 (1998) 207-212
32. T. A. Becker, D. R. Kipke and T. Brandon, Calcium alginate gel: a biocompatible and mechanically stable polymer for endovascular embolization, *J Biomedical Materials Research.* 54(1) (2002) 76–86.
33. W. R. Gombotz and S. F. Wee, Protein release from alginate matrices. *Adv. Drug Deliv. Rev.* 31(1–2) (1998) 267–285.
34. D. A. Rees, Polysaccharide shapes and their interactions, *Some recent advances. Pure and Applied Chemistry.* 53 (1981)1–14.
35. Y. S. Khotimchenko, V. V. Kovalev, O. V. Savchenko and O. A. Ziganshina. Physical–chemical properties, physiological activity, and usage of alginates, the polysaccharides of brown algae, *Russian Journal of Marine Biology.* 27(1) (2001) S53–S64.
36. P. Sriamornsak, S. Prakongpan and S. Puttipipatkachorn, Calcium pectinate gel coated pellets as an alternative carrier to calcium pectinate beads, *Int. J. Pharm.* 156 (1997) 189–194.
37. P. Sriamornsak and J. Nunthanid, Calcium pectinate gel beads for controlled release drug delivery: I. Preparation and *in vitro* release studies, *Int. J. Pharm.* 160 (1998) 207–212.
38. T.W. Wong, H.Y. Lee, L.W. Chan and P.W.S. Heng, Drug release properties of pectinate microspheres prepared by emulsification method, *Int. J. Pharm.* 242 (2002) 233–237.
39. V. U. Rao, M. Vasudha, K. Bindu, S. Samanta, P. S. Rajinikanth, B. Mishra and J. Balasubramaniam, Formulation and *in vitro* characterization of sodium alginate–gellan beads of glipizide, *Acta Pharmaceutica Scientia.* 49 (2007) 13–28.
40. H. Y. Lee, L. W. Chan, A. V. Dolzhenko and P. W. S. Heng, Influence of viscosity and uronic acid composition of alginates on the properties of alginate films an microspheres produced by emulsification, *J. Microencapsulation.* 23(8) (2006) 912–927.
41. X. D. Liu, W. Y. Yu, Y. Zhang, W. M. Xue, W. T. Yu, Y. Xiong, X. J. Ma, Y. Chen and Q. Yuan, Characterization of structure and diffusion behaviour of Ca–alginate beads prepared with external or internal calcium sources, *J Microencapsulation.* 19(6) (2002) 775–782.
42. H. H. Tønnesen and J. Karlsen, Alginate in drug delivery systems, *Drug Dev Ind Pharm.* 28 (2002) 621Y630.
43. H. Grasdalen and O. Smidsroed, Gellation of gellan gum, *Carbohydr. Polym.* 7 (1987) 371–393; DOI: 10.1016/0144-8617(87)90004-X.
44. R. Chandrasekaran, L. C. Puigianer, K. L. Joyce and S. Arnotts, The influence of calcium ions, acetate and L-glycerate groups on the gellan double-helix, *Carbohydr. Res.* 181 (1988) 23–40; DOI: 10.1016/0008-6215(88)84020-5.
45. R. Chandrasekaran and V. G. Thailambad, Cation interactions in gellan: An x-ray study of the potassium

- salt, *Carbohydr. Polym.* 12 (1990) 431–442; DOI: 10.1016/0144-8617(90)90092-7.
46. C. Sartori, D. S. Finch and B. Ralph, Determination of the cation content of alginate thin films by FTIR spectroscopy. *Polymer*, 38 (1997) 43–51.
47. N. P. Chandia, B. A. Matsuhira and E. Vasquez, Alginic acids in *Lessonia trabeculata*: characterization by formic acid hydrolysis and FT-IR spectroscopy, *Carbohydrate Polymers*, 46(1) (2001) 81–87.



***Corresponding Author:**

Ramya Krishna. T *

St. Peter's Institute of Pharmaceutical Sciences,
Hanamkonda, Warangal, A.P, India