Available online at http://www.tjpr.org

Research Article

Formulation and *In Vitro* Evaluation of pH-Sensitive Oil-Entrapped Buoyant Beads of Clarithromycin

GK Tripathi* and S Singh

Industrial Pharmacy Laboratory, Department of Pharmacy, Saroj Institute of Technology & Management Lucknow, India-226001

Abstract

Purpose: *To develop pH-sensitive controlled release formulation of clarithromycin in oil-entrapped calcium pectinate microgel bead.*
Methods: *Pectin-based oil-entrapped microgel beads were prepared by ionic gelation technique. The gel beads were formed instantly after adding the liquid formulation mixture dropwise into calcium chloride solution. The beads were optimized by coating with ethylcellulose solution and then evaluated for their diameter, floating lag time, encapsulation efficiency and drug release.*
Results: *Particle size, encapsulation efficiency and buoyancy were significantly affected by the concentration of the polymer and calcium chloride .The formulation exhibited sustained release profile and was best fitted to the Peppas model with n < 0.45. Ethylcellulose-coated formulation batch, C_{16}, was the most suitable controlled formulation with drug release of 65 ± 2.61 % in 8 h.*
Conclusion: *An ethylcellulose-coated formulation with potential for sustained delivery of clarithromycin in the acidic region of the gastrointestinal tract was successfully developed.*

Keywords: *Clarithromycin; Calcium pectinate bead; Gastric residence time; pH-sensitive; Ethyl cellulose; Oil-entrapped*

---

*Corresponding author: **E-mail:** orgpharm@gmail.com; **Tel:** 09415745934*

INTRODUCTION

The oral route has attracted special attention for the delivery of anti infective agents that are needed to produce local action in the gastrointestinal tract. This route of administration usually shows high compliance by patients due to ease of administration.

A search of the scientific and patent literature reveals an increased interest in novel dosage forms for the targeting of different parts of the gastrointestinal tract for drug retention at the site of action for a predetermined time [1,2]. This approach is especially most attractive for the delivery of anti-infective agents for the targeting of local microbial lesions inside gastrointestinal tract and promises to provide a more effective cure of such infectious lesions than conventional dosage forms. This is because a major problem frequently encountered with conventional oral dosage forms is the inability to localise drug release in the stomach and proximal portion of the small intestine [3,4]. Floating drug and bioadhesive drug delivery systems are widely used techniques for gastroretention [5,6].

Clarithromycin is a semi-synthetic macrolide antibiotic derived from erythromycin. It is primarily bacteriostatic and exerts its antimicrobial effect by the inhibition of protein synthesis on bacterial ribosome [7,8]. Pectin is a colloidal polygalacturonic acid in which some of the carboxylic groups are esterified with methyl groups. The main constituent of pectin is D-galectouronic acid [9,10]. This low methoxy polysaccharide polymer, with a degree of esterification < 50 %, can form rigid gels in the presence of calcium ions or other multivalent cations which crosslink the galacturonic acid chains of pectin to yield hydrogels 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 emulsions [11].

The objective of this work was to develop a gastroretentive, multiple-unit, controlled release formulation of clarithromycin that would achieve continuous release of the drug in the gastric region and thus be useful for complete termination of microbial infection at gastric sites.

EXPERIMENTAL

Materials

Clarithromycin was obtained as a gift from Ranbaxy Laboratories, Devash, India. Low-methoxy pectin, with a degree of esterification of 35 %, and ethyl cellulose, were obtained from S.D. Fine Chemicals, India. Light mineral oil and castor oil were supplied by Central Drug House, India.

Preparation of calcium pectinate beads

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

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

Formulation	Pectin (%w/v)	Oil (%w/v)	CaCl$_2$ (mol/L)
C$_1$/M$_1$	1.25	5	0.275
C$_2$/M$_2$	1.25	10	0.275
C3/M$_3$	1.85	5	0.275
C$_4$/M$_4$	1.25	5	0.450
C$_5$/M$_5$	1.25	15	0.275
C$_6$/C$_6$	1.85	5	0.450
C$_7$/C$_7$	1.25	15	0.450
C$_8$/C$_8$	1.85	10	0.450
C$_9$/C$_9$	1.85	15	0.450

Note: *C and M are formulations containing castor oil and mineral oil, respectively; each formulation contained clathromycin 0.5 %w/v*

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 %) with continuous stirring until a uniform dispersion containing 0.5 % of the drug was obtained. The mixture was emulsified with either mineral oil or castor oil using a Silverson emulsifier (Hicon, India) at a constant stirring rate of 500 rpm for 5 min. The resulting drug-loaded emulsion was dropped through a 21G syringe needle separately into 100 mL of 0.275 - 0.45 mol ml^{-1} of calcium chloride solution and stirred with a magnetic stirrer to improve the mechanical strength of the beads and to prevent their aggregation. Formation of small microgel beads of clarithromycin based on either castor oil (COB) or mineral oil (MOB) occurred after 5 min of curing. The beads were washed with distilled water, collected by filtration throughWhatman filter paper no. 1 and dried in a tray dryer at 40 °C for 6 h.

Coating of gel beads

The selected gel bead formulations were coated with ethyl cellulose (EC) in a 2^2 factorial design (see Table 2) for optimization. The coating formulation was 5 – 10 %w/v ethyl cellulose (EC) solution in acetone and coating time was 5 - 10 min. The gel beads (2 g) were placed in a fluidized bed dryer (TG 100, Retsch, Germany) and the fluidized beads were sprayed with the coating solution for a period of 5 or 10 min at an air inlet speed of 220 m/s at room temperature. The beads were dried at room temperature for a period of 24 h when the solvent evaporated, leaving a film coat on the gel beads.

Size and morphology

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

In vitro floating test was performed using a USP 24 dissolution apparatus II in 500 mL of phthalate buffer solution (pH 3.4) with the medium temperature kept at 37 ± 0.5 °C. The floating beads (1 g) were placed in the dissolution medium 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 were observed for up to 12 h [12].

Determination of drug-loading and encapsulation efficiency

Accurately weighed (100 mg) grounded powder of beads was soaked in 100 ml phosphate buffer (pH 7.5) and allowed to disintegrating completely for 4 h [13]. 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 λ_{max} of 353 nm against a constructed calibration curve. The encapsulation efficiency (EE) was calculated according to the relationship in Eq 1.

EE (%) = (C/T) x 100 ………………..………. (1)

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 the gel beads using USP 24 dissolution test apparatus II (basket type) [13]. Accurately weighed 50 mg amount of the bead

(containing 19 – 21 mg of active drug) dropped in 900 ml of simulated gastric fluid (SGF, fasting state, pH 1.2; prepared by dissolving 2 g of sodium chloride, 3.2 g pepsin, and 6.8 ml of hydrochloric acid in double distilled water to 1 L), 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 h, 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 353 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 containing 10 mg clarithromycin and lactose (q.s.) filled into an empty capsule shell (#2) was used as a reference formulation.

Kinetic release evaluation

To investigate the mode of drug release from the microgel beads, the release data were analyzed with various release kinetic models (zero order, Higuchi and Korshmaer-Peppas) were applied to elucidate their mechanism of drug release in the fed state [14-16]. The analysis of the dissolution data was carried out using Eqs 2 – 4 for zero order, Higuchi and Korsemeyer-Peppas models, respect-tively.

$$M_t = M_0 + K_0 t \ \text{……………………...……….} \ (2)$$
$$M_t = M_0 + K_H t^{0.5} \ \text{………………………..} \ (3)$$
Korsemeyer-Peppas model:
$$M_t/M_\infty = kt^n \ \text{………………………………} \ (4)$$

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.

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 test was also applied. Statistically significant difference between the means of batches was set at $p < 0.05$.

RESULTS

The scanning electron micrographs (SEM) of the dried microgel beads, C_6 and M_6, are shown in Figure 1. Gel beads prepared from mineral oil (MOB) were white, translucent and rigid, while castor oil-based gel beads (COB) were off–white, translucent and elastic.
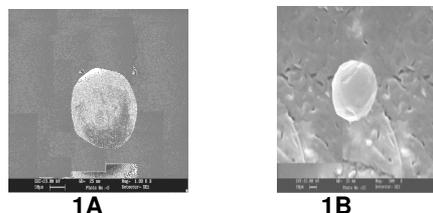

1A   **1B**

Figure 1: Scanning electron micrographs of dried beads of batch C_6 (COB, 1A) and batch M_6 (MOB, 1B)

The diameter of MOB varied between 1.24 ± 0.60 and 1.46 ± 0.30 mm while that of COB was between 1.45 ± 0.80 and 1.80 ± 0.20 mm. Floating lag time (the time taken for the beads to float at the surface of the medium) was 10 – 52 s and 16 -74 s for MOB and COB, respectively (Table 3).

Encapsulation efficiency was highest for batch C_6 (75.0 ± 0.8 %) and batch M_6 (70.0 ± 0.3 %) while drug content was 55.0 ± 1.3 % and 67.0 ± 1.3%, respectively, for the batches.

In vitro drug release

The dissolution data are shown in Fig 2. The results indicate that 90.1 ± 2.5 % of the pure drug (batch F) dissolved in 2 h in fasting state

(pH 1.2) while for the fed state (F_2, pH 3.4), the figure was 87.5 ± 2.5 %. In 4h, drug release from batch C_6 was 80.0 ± 2.4 % (fasting state) and from batch M_6 76.0 ± 2.7 % (fed state) in simulated gastric fluid (SGF). For the EC-coated beads (batch C_{61}), a maximum dissolution efficiency of 65.5 % was attained in 8 h, as shown in Table 2.

Table 2: Independent variables for ethyl cellulose-coated beads

Formulation code	EC conc. (%)	Coating Time (min)	DR_{8h} (%)	R^2
C_{61}	5	5	65.5	0.984
C_{62}	5	10	60.4	0.972
C_{63}	10	5	50.8	0.972
C_{64}	10	10	42.4	0.968

DR = dissolution efficiency; R^2 = correlation coefficient

Drug release from the optimized bead formulations C_6 and M_6 followed the Higuchi (R^2 = 0.9841, n = 0.41) and Peppas models (R^2 = 0.9827, n = 0.39), respectively. Correlation coefficient (R^2) of the coated batches (see Table 2), based on zero order release kinetics, ranged from 0.968 – 0.984.

DISCUSSION

Spherical gel beads were formed instantaneously when emulsion was dropped into calcium chloride solutions. Gelation occurred due to intermolecular cross-linking between the divalent calcium ions and the negatively charged carboxyl groups of pectin. Pectin promoted the emulsification of the mixture of water and oil phases during homogenization and the resulting oil droplets were dispersed in calcium crosslinked network of the formulation.

Table 3: Characterization of 100 mg clarithromycin gel beads (± SD, n = 3 or 100)

Formulation		Diameter (mm)		Lag time (s)		Encapsulation efficiency (%)		Drug content (%)	
COB	MOB	MOB	COB	MOB	COB	MOB	COB	MOB	COB
C_1	M_1	1.24±0.6	1.45±0.8	20±1.6	30±1.3	58±0.6	60±0.8	41±1.2	52±2.1
C_2	M_2	1.32±0.5	1.53±0.7	28±1.3	37±1.8	52±0.3	56±0.4	56±1.3	47±1.5
C_3	M_3	1.36±0.3	1.58±0.2	46±1.4	57±1.6	62±0.3	68±0.3	48±1.3	60±1.3
C_4	M_4	1.28±0.7	1.49±0.6	15±1.9	26±1.5	55±0.4	58±0.8	39±1.3	50±1.2
C_5	M_5	1.34±0.8	1.55±0.4	36±1.8	47±1.8	59±0.7	50±0.5	51±1.3	42±1.5
C_6	M_6	1.46±0.3	1.67±0.5	10±1.7	16±1.9	70±0.8	75±0.8	55±1.3	67±1.3
C_7	M_7	1.38±0.2	1.59±0.3	40±1.8	58±1.2	61±0.9	62±0.2	43±1.3	54±1.8
C_8	M_8	1.39±0.3	1.60±0.7	48±1.3	66±1.3	53±0.9	64±0.7	45±1.2	56±1.7
C_9	M_9	1.40±0.4	1.80±0.2	52±1.2	74±1.9	60±0.7	62±0.9	43±1.2	54±1.3
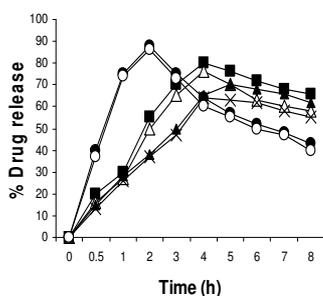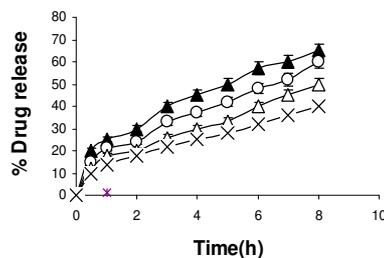
2A	2B

Figure 2: Comparative drug release profile for fasting states (2A) (●=F_2, ■ = C_6, Δ = M_6); and fed state (2B) (▲ = FC_6, x = FM_6, ○ = F); and for fed state of ethyl cellulose-coated beads (Δ = C_{63}, X = C_{64})

The diameter of the beads increased significantly ($p < 0.05$) as polymer concentration increased; this could be attributed to the increase in the micro-viscosity of the polymeric dispersion, eventually leading to the formation of larger beads. Larger size beads were also formed as the concentration of calcium chloride increased. This may be due to excess calcium ions causing possibly all the crosslinking sites in the polymer to be fully utilized and resulting in larger but weaker and flexible gel beads.

Buoyancy is an important factor in sustained drug delivery to the gastric region. All the beads floated on simulated gastric fluid for up to 12 h. Increase in the calcium chloride content of the beads resulted in a decrease in floating lag time. due to increase in the porosity of the gel beads. Floating lag time also rose as the concentration of oil in the formulation increased and this can be attributed to flocculation of the oil globules which might also have coalesced to produce large droplets.

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.

Drug release from the beads (as shown in Fig 2A) was characterized by an initial phase of rapid release ('burst effect') due to the presence of clarithromycin on the bead surface since the drug exhibits good solubility at low pH [17].. However, release thereafter slowed down due to the obstruction of drug diffusion by pectin - Ca^{2+} ions crosslinks. The release exponent (n) value of 0.045 suggests a diffusion-based release mechanism [18].

However, the dissolution profiles of the coated beads (batch C_{62}, C_{62}, C_{63} and C_{64}) were best fitted to the zero-order kinetic model (Fig 2B) . Formulation batch C_{61} was considered the optimized gastroretentive controlled-release floating gel bead for clarithromycin, as it showed the lowest release.

CONCLUSION

The developed oil-entrapped gel beads showed good floating and controlled drug release properties at simulated acid pH conditions of the stomach. Therefore, it may be capable of delivering clarithromycin to stomach sites, thus opening up the possibility of targeting the drug to gastric sites for the treatment of microbial infections such as that caused by *H. pylori*.

ACKNOWLEDGEMENT

REFERENCES

1. Jain SK, Manmohan S. Lectin Conjugated Gastroretentive Multiparticulate Delivery System of Clarithromycin for the Effective Treatment of Helicobacter pylori. Mol Pharmaceutics 2009; 6 : 295-304.
2. Roy SK, Prabhakar B. Bioadhesive Polymeric Platforms for Transmucosal Drug Delivery Systems – a Review. Trop J of Pharm Res 2010; 9: 91-104.
3. Rouge N, Buri P, Doelker E. Morphology and buoyancy of oil-entrapped calcium pectinate gel beads. Int J Pharm 1996; 136: 117–139.
4. 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.
5. Choi BY, Park HJ, Hwang SJ, Park JB. Preparation of alginate beads for floating drug delivery

system: effects of CO_2 gas-forming agents. Int J Pharm 2002; 239: 81-91.

6. Niagara 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.

7. Rajinikanth PS, Mishra B. Floating in situ gelling system for stomach site-specific delivery of clarithromycin to eradicate H. pylori. J Contr Rel 2008; 125: 33–41.

8. Rajinikanth PS , Mishra B . Preparation and in vitro characterization of gellan based floating beads of acetohydroxamic acid for eradication of H. pylori. Acta Pharm 2007; 57: 413–427.

9. Rolin C, Whistler RL, Bemiller JN . Industrial Gums: Polysaccharides and their derivatives. New York, Academic Press 1993; pp 257-293.

10. Schols HA, Voragen AG. Complex pectin: structure elucidation using enzymes. In: Visser J, Voragen AGJ, Eds. Progress in Biotechnology: Pectin and Pectinases. Amsterdam, the Netherlands, Elsevier, 1996; pp 3-19 .

11. Leroux J, Langendorff V, Schick G, Vaishnav V. Emulsion stabilizing properties of pectin. Food Hydrocolloids 2003; 17: 455-462.

12. Cooreman MP, Krausgrill P, Hengels KJ . Local gastric and serum amoxicillin concentrations after different oral application forms. Antimicrob Agents Chemotherm 1998; 37: 1506–1509.

13. United State Pharmacopeia 2002, 24/National Formulary 19, USP Convention. Rockville, MD.

14. Wagner JG. Interpretation of percent dissolved-time plots derived from in vitro testing of conventional tablets and capsules. J Pharm Sci 1969; 58: 1253–1257.

15. 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.

16. Korsmeyer RW, Gurny R, Peppas N. Mechanisms of solute release from porous hydrophilic polymers . Int J Pharm 1983; 24: 25–35 .

17. Ling HQ, Leng WL. Formulation and evaluation of less-painful clarithromycin lipid microspheres. J Pharm 2003; 250: 403-414.

18. Streubel A, Siepmann J, Bodmeier R. Floating microparticles based on low density foam powder. Int J Pharm 2002; 241: 279–292.