



DESIGN AND CHARACTERIZATION OF CLARITHROMYCIN BEADS USING A BIODEGRADABLE CATIONIC POLYMER

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ABSTRACT

The objective of the present study was to prepare clarithromycin beads by ionotropic gelation method using cationic polymer. Infra red spectroscopy study showed that the drug and excipients were compatible with each other. The beads were evaluated for SEM, average particle size, swelling studies, zeta potential, drug entrapment and in vitro drug release. The average particle size of the beads was between 0.87 ± 0.020 and 1.254 ± 0.030 mm. The swelling ratio was greater in buffer pH 1.2 and the zeta potential values showed positive charge of the beads. The *in vitro release* study showed that the drug release was found to follow first order diffusion controlled release.

KEYWORDS: Clarithromycin, Cationic, Chitosan, Beads

INTRODUCTION

In a relatively short span, *Helicobacter pylori* (*H. pylori*) have become recognized as a major gastric pathogen with world-wide distribution. *H. pylorus* is spiral-shaped bacterium found in the stomach, which (along with acid secretion) damages stomach and duodenal tissue, causing inflammation and peptic ulcers. This prevalent human-specific pathogen is a causative agent in chronic active gastritis¹ and duodenal ulcers² and gastric adenocarcinoma³, one of the most common forms of cancer in humans. It resides mainly in the gastric mucosa or at the interface between the mucous layer and epithelial cells of the antral region of the stomach⁴.

Clarithromycin is a semi synthetic, macrolide antibiotic which is stable in gastric environment and it is absorbed rapidly from gastrointestinal tract after oral administration. It is widely used in standard eradication treatment of gastric *H. pylori* infection combined with an acid-suppressing agent and a second antibiotic^{5, 6}. It is a US FDA approved drug regimen for the treatment.

Chitosan (obtained by deacetylation of chitin) is a cationic polymer, posses' antacid and antiulcer activity⁷, mucoadhesive properties⁸⁻¹³ due to molecular attractive forces formed by electrostatic interaction between oppositively charged chitosan and negatively charged mucosal surfaces. It is a biodegradable natural polymer with high charge density, non-toxic and biocompatible¹⁴.

The present work was carried out with an aim to prepare clarithromycin beads using chitosan as a biodegradable natural polymer and to study the characterization of the beads.

MATERIALS AND METHODS

Clarithromycin U.S.P was obtained as a gift sample from Biochem Pharmaceutical Industries Ltd, Mumbai. Chitosan (85% degree of deacetylation, molecular weight 10^3 KD) was obtained as a gift sample from Central Institute of Fisheries, Cochin, India. Sodium tri polyphosphate (TPP) was purchased from Merck, Sigma chemicals, USA. Simulated gastric fluid (S.G.F) pH 1.2 and simulated intestinal fluid (S.I.F) pH 7.5 were prepared as prescribed in US Pharmacopoeia (USP XII 1995). All other solvents and

reagents were of analytical grade and used without further purification.

IR Spectroscopic analysis

The IR spectroscopic analysis was performed to study any interaction between the drug and the excipients used in the preparation. IR spectra of the drug and the beads were recorded in FT/IR-410 JASCO spectrophotometer using KBr pellets.

Preparation of Clarithromycin Beads

Clarithromycin beads were prepared by ionotropic gelation method¹⁵. Chitosan was selected as a polymer due to its cationic and biodegradable properties. Chitosan showed good solubility in aqueous acid. Polymer concentrations of 1.0%, 1.5%, 2% w/v and Sodium tripolyphosphate concentrations of 0.5%, 1%, 2% w/v were used for the study. Chitosan solution was too viscous to drop when the concentration was above 2% w/v. Therefore 1%, 1.5%, 2% w/v was selected for this study shown in Table No: 1. Sodium tripolyphosphate was used as ionic crosslinking agent and the volume (100ml) was kept constant throughout the study. Glutaraldehyde was also used as a crosslinking agent (chemical) during the formation of beads and its effect on morphology of the prepared beads were studied.

Characterization of prepared beads

Particle Size Determination

The particle size of beads was carried out using optical microscope along with stage micrometer and the average diameters of 100 beads were calculated in each batch using calibration factor,

$$X = \sum (x_i) / N$$

Scanning Electron Microscopy

The surface morphology of beads was studied by using Jeol JSMC-2500 model scanning electron microscopy (SEM). The beads were mounted on an appropriate stub and then gold coated under vacuum. The gold coated samples were then observed under scanning electron microscope.

Surface Charge Measurements

The surface charge on control and chitosan beads containing clarithromycin were determined by Zeta sizer (NANO ZS, ZEN 3600, MALVERN Company) in de ionized water and in S.G.F pH 1.2 and in S.I.F pH 7.4.

Determination of Drug Load

Clarithromycin content in beads were determined by digestion method. Drug loaded beads (150mg) were pulverized and incubated in HCl buffer pH 1.2. The suspension was sonicated and vibrated in a water bath for 150 min. Then, it was cooled to room temperature. The supernatant was assayed spectrophotometrically for clarithromycin content at 210 nm. Supernatant from empty beads (without drug) was taken as a blank. All the samples were analyzed in triplicate.

Equilibrium Swelling Studies

A known weight (100mg) of clarithromycin beads (prepared with 2% chitosan) from formulations F3, F6, F9 and F12 were placed in various solutions like deionized water, enzyme-free simulated gastric fluid (HCl / NaCl solution; pH 1.2) and enzyme-free simulated intestinal fluid ((KH₂PO₄) NaOH buffer; pH 7.4), allowed to swell for 4 hours at 37 ± 0.5°C. The beads were periodically removed, blotted with filter paper and their change in weight was measured until equilibrium swelling was attained. Swelling ratio (SR) was calculated for swollen beads using the formula,

$$SR = (W_c - W_o) / W_o$$

Where W_o is the initial weight of dry beads and W_c is the weight of the swollen beads after equilibrium swelling. The experiment was repeated three times and the average value ± S.D was taken as SR.

Table 1: Formulation variables for clarithromycin beads

Formulation Code	Polymer Concentration (%w/v)	TPP Concentration (%w/v)
F1 ⁺	1.0	1.0
F2 ⁺	1.5	1.0
F3 ⁺	2.0	1.0
F4	1.0	0.5
F5	1.5	0.5
F6	2.0	0.5
F7	1.0	2.0
F8	1.5	2.0
F9	2.0	2.0
F10	1.0	1.0
F11	1.5	1.0
F12	2.0	1.0

(+) 25%v/v of aqueous glutaraldehyde solution (1ml)

Table 2: Average zeta potential values for clarithromycin beads

Formulation Code	Zeta potential (mV)
A	8.37±0.21
B	5.72±0.12
C	16.24±0.86
D	11.36±0.07

A= chitosan beads without drug; B= chitosan beads with drug; C= crosslinked beads without drug; D= crosslinked beads with drug

Table 3: Determination of equilibrium swelling ratio of clarithromycin beads

Formulation Code ⁺	Deionized water	S.G.F pH 1.2	S.I.F pH 7.4
F3	0.75±0.11	1.26±0.15	0.45±0.18
F6	0.360±0.054	0.56±0.21	0.47±0.18
F9	0.04±0.01	0.18±0.02	0.16±0.04
F12	0.811±0.014	2.85±0.28	0.91±0.20

(+) 2%w/v of chitosan solution

Table 4: In vitro release kinetics of clarithromycin from chitosan beads

Formulation Code	Co. efficient of variation 'r' values		
	Zero order	First order	Higuchi equation
F1	0.9836	0.9886	0.9975
F2	0.9656	0.9919	0.9965
F3	0.9706	0.9925	0.9940
F4	0.9938	0.9735	0.9899
F5	0.9648	0.9685	0.9954
F6	0.9604	0.9808	0.9937
F7	0.9821	0.9278	0.8629
F8	0.9648	0.9696	0.9953
F9	0.9804	0.9855	0.9949
F10	0.9745	0.9696	0.9961
F11	0.9685	0.9927	0.9953
F12	0.9643	0.9886	0.9956

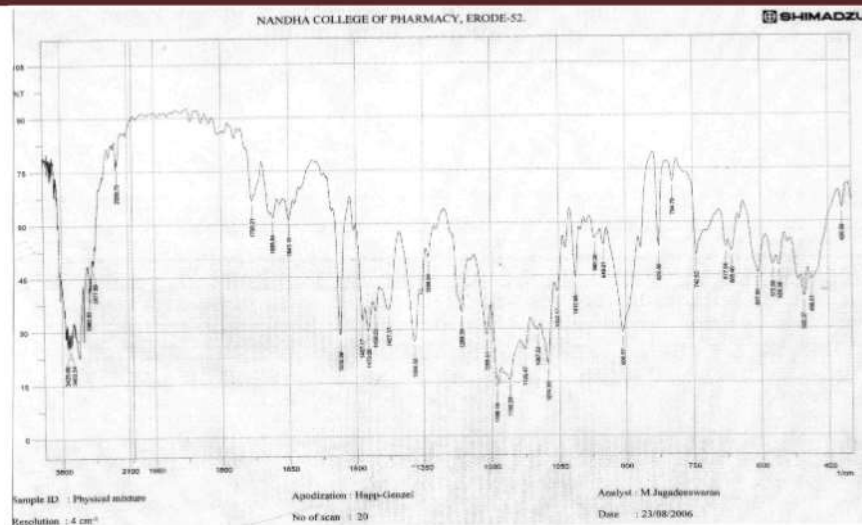


Figure: 1 FTIR spectral analysis of drug and excipients physical mixture

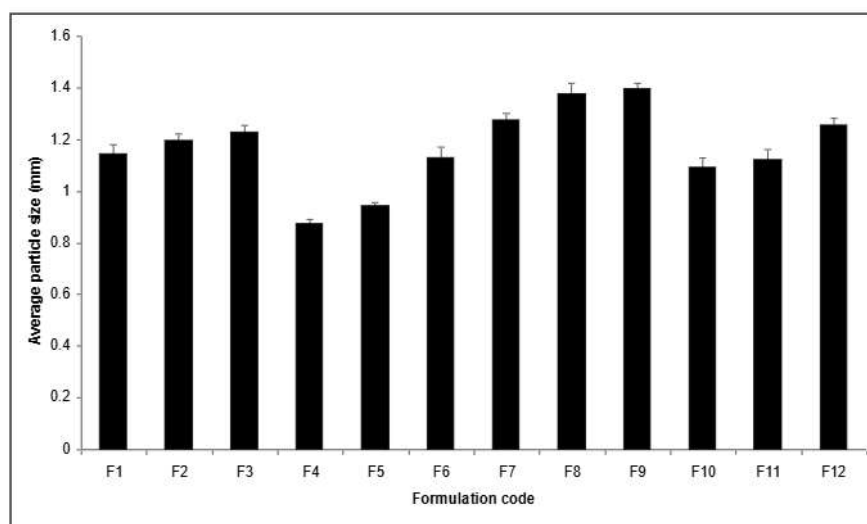


Figure: 2 Average particle size of clarithromycin beads prepared using chitosan. Bars represent mean $\pm$ S.D. (n=3)

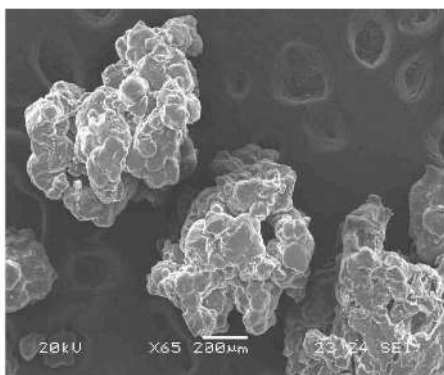


Figure: 3(a) SEM photographs of clarithromycin beads prepared with 2% TPP

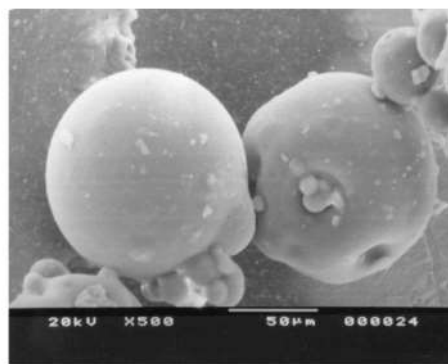


Figure: 3(b) SEM photographs of clarithromycin beads prepared with aqueous glutaraldehyde solution

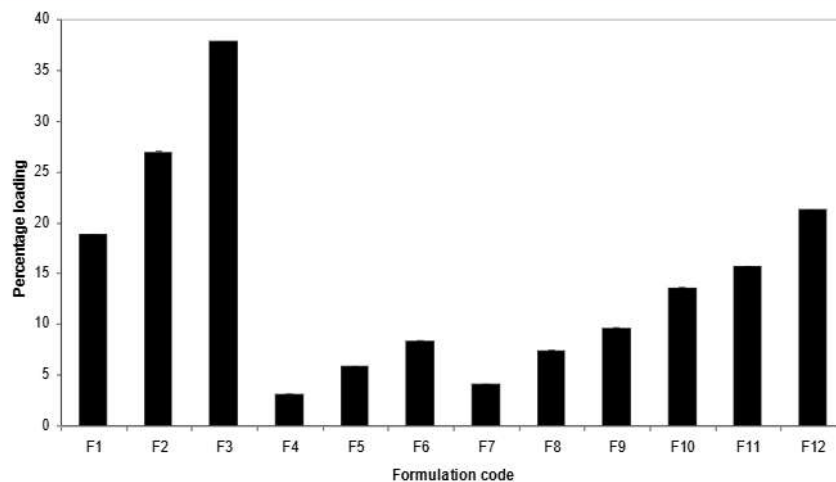


Figure: 4 Percentage of clarithromycin loaded in chitosan beads. Bars represent mean $\pm$ S.D. (n=3)

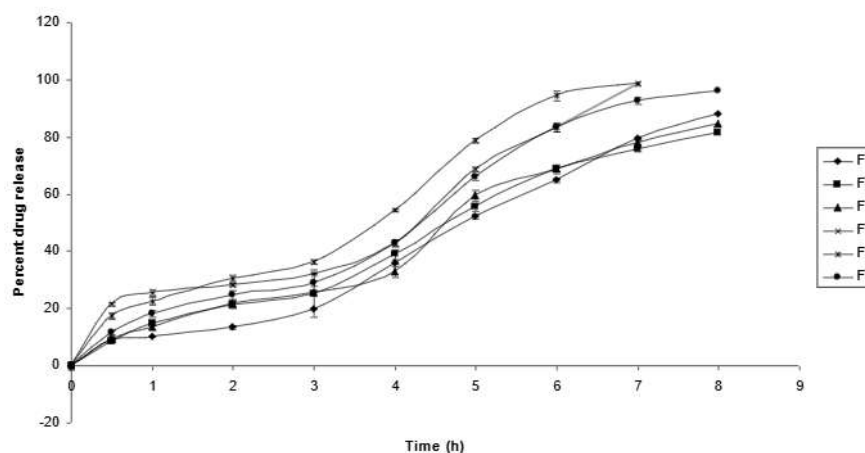


Figure: 5 Percentage of clarithromycin released from formulations F1 to f6 as a function of time at 37°C in S.G.F (pH 1.2). Bars represent mean $\pm$ S.D. (n=3).

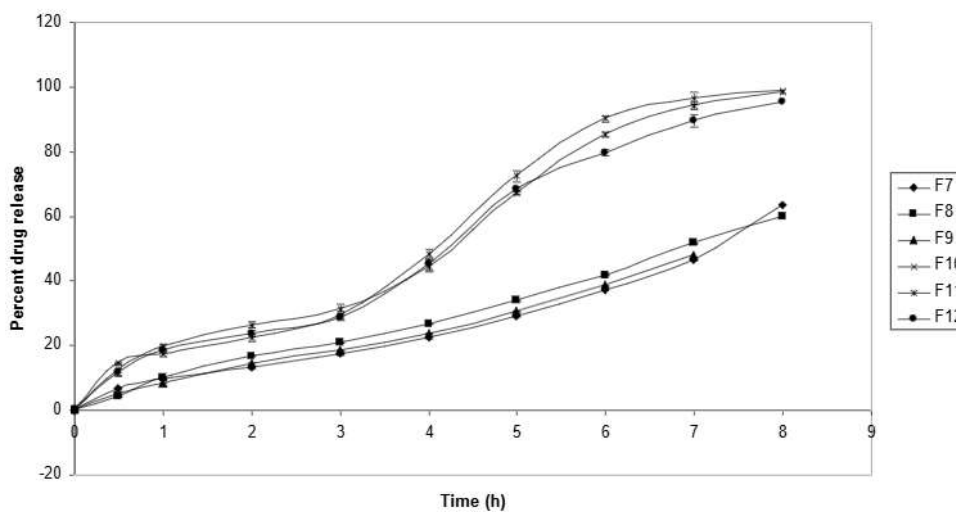


Figure: 6 Percentage of clarithromycin released from formulations F7 to F12 as a function of time at 37°C in S.G.F (pH 1.2). Bars represent mean $\pm$ S.D. (n=3)

In Vitro Release Study

The release rate of clarithromycin from chitosan beads were studied using USP dissolution testing apparatus I (basket method). The dissolution test was performed using 900 ml HCl buffer pH 1.2 at $37 \pm 0.5^\circ\text{C}$ with an agitated speed of 100 rpm. About 5 ml of sample was withdrawn at appropriate time intervals, filtered through 0.45mm membrane filter and the absorbance was measured spectrophotometrically at 210 nm. The volume withdrawn was replaced with fresh medium and adjusted to 900ml every time. All the batches were studied in triplicate.

RESULTS

The physical mixture of drug and polymer was characterized by FTIR spectral analysis for any significant interaction between the drug and the excipients used during the formulation. From the results, it was evident that there was no interference in the functional groups and the principle peaks of clarithromycin were found to be unaltered in the drug-polymer physical mixture, indicating they were chemically compatible, which was shown in Figure 1.

Clarithromycin beads were prepared by ionotropic gelation method using chitosan as a polymer which were shown in Table 1. The average particle size of clarithromycin beads ranges between $0.87 \pm 0.020\text{mm}$ and $1.394 \pm 0.025\text{mm}$ were shown in Figure 2. The particle size was greater for batches prepared with high concentration of TPP (2%) but showed irregular beads [Figure 3(a)]. Formulations (F1, F2, and F3) those prepared with aqueous glutaraldehyde solution showed uniform spherical particles Figure 3(b). Formulations (F4, F5, and F6) showed flat irregular particles (upon drying).

The surface charge for drug loaded and unloaded beads were measured using zeta sizer in deionized water, S.G.F pH 1.2 and in S.I.F pH 7.4. The average zeta potential for drug loaded and unloaded beads were +16.24mV and +8.37mV respectively which were shown in Table 2.

Figure 4 showed that the drug content was increased up to 37.83 % upon addition of aqueous glutaraldehyde solution. A low drug entrapment was observed when the concentration of TPP was kept low (0.5%) but the high TPP concentration (2%) does not showed much deviation in drug content of the clarithromycin beads.

Swelling ratio was conducted up to 4 hours in S.G.F pH 1.2, S.I.F pH 7.4 and in deionized water. The results indicated that the degree of swelling was influenced greatly by pH of the medium, greater swelling was observed at lower pH value (S.G.F) than in S.I.F and in de ionized water. The study also reveals that the concentration of polymer have significant role in determining the swelling character of bead. Formulation F3 showed reduced swelling than F9 and F12 but F6 could not maintain the shape and formed gel due to low TPP concentration which was shown in Table 3.

The in vitro drug release was studied using S.G.F pH 1.2 at $37 \pm 0.5^\circ\text{C}$ shown in Figure 5 and 6. Formulations prepared with low TPP concentrations (F4, F5 and F6) released almost 98.9% within 7 hours whereas F2 showed 96.2% at 8 hours. Drug release was faster for formulations F10, F11, F12 compared to F1, F2 and F3. Therefore from the above study it was understood that those batches (F1, F2, and F3) prepared with glutaraldehyde were able to sustain the drug release for longer time than those prepared without glutaraldehyde. The release data were fitted to models representing zero order,

first order and Higuchi's square root of time. The linear regression analyses were summarized in Table 4.

DISCUSSIONS

In conclusion, clarithromycin beads prepared with cationic polymer has the possibility of sustaining the drug release over the period of at least 8 hours to increase the local concentration of drug in the stomach. The coefficient of determination of 'r' values for different formulations indicated that the drug release from clarithromycin beads followed a first order diffusion controlled mechanism of drug release.

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