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Research Article

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Clarithromycin Loaded Floating Eudragit Microsphere for Anti *H. Pylori* Therapy: *In-vitro* and *In-vivo* Assessment

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ABSTRACT

The objective of present research work was to develop floating microspheres for oral delivery of clarithromycin for treatment of Helicobactor pylori to provide prolonged contact time of to the stomach. Floating microsphere were prepared by solvent diffusion and evaporation method and characterized for particle size, % entrapment efficiency, % buoyancy and surface morphology by using scanning electron microscopy (SEM). Prepared formulation has particle size 142.26 nm, entrapment efficiency 79.22% and buoyancy 93.35%. In vitro drug release study and in vivo radioimaging study also carried out. The buoyancy and release profile of the floating microspheres shown have potential for delivery of clarithromycin to the stomach. These microspheres resided in the stomach upto 6 hour for treatment of H. pylori.

Keywords: Floating microsphere; *Helicobactor pylori;* Buoyancy; Clarithromycin

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INTRODUCTION

Helicobacter pylori, a prevalent human-specific pathogen, was discovered in 1982, confirmed as a pathogen at the end of the1980's and classified as type I carcinogen in 1994 by the World Health Organization (WHO) [1]. *H. pylori* has been regarded as a major human gastric pathogen for chronic active gastritis, peptic ulcers, gastric adeno-carcinoma and gastric cancer in human, because it can easily colonize human gastric and duodenal mucosa as well as attached to mucus cells [2,3]. Recently, a triple therapy consisting of two antibiotics (amoxicillin, metronidazole or clarithromycin) and a proton-pump inhibitor was recommended to treat against *H. pylori* infections [4]**.**

Treatment of *H. pylori* remain a challenge although *H. pylori* is highly sensitive to most antibiotics, eradication of *H. pylori* from patient is difficult with conventional dosage forms because not retained in the stomach for long periods [5]; therefore it is difficult to achieve minimum inhibitory concentrations (MIC) in the gastric mucosa where the *H. pylori* reside [6]. Gastroretentive dosage forms have the potential to improve local therapy against gastric diseases with an increase of short gastric residence time and unpredictable gastric emptying time and decrease the variation in bioavailability [7]**.** The failure in gastric retention with conventional systems has led to the development of oral gastroretentive systems. Such delivery systems were designed to be retained in the upper gastrointestinal tract for a prolonged period of time, during which they release the drug on a controlled basis [8]. Various carrier systems studied to deliver clarithromycin for treatment of *H. pylori* poly (lactic-co-glycolic acid) nanospheres [9] and chitosan hydrochloride-genipin crosslinked microspheres [10], liposomes [11] but not perform *in vivo* study. The aim of this study was to prepare clarithromycin (CTM)-loaded Eudragit S 100 microspheres and clarithromycin (CTM) was selected drug, which is widely used in combination therapy for *H. pylori* infection. Eudragit is frequently used in the coating of solid dosage forms, which is insoluble in aqueous media. In the present study, we prepared porous eudragit microspheres and evaluate for the physicochemical characteristics were examined using particle size, entrapment efficiency, surface morphology, *in vitro* drug release, stability study and *in vivo* radiographic studies.

MATERIALS AND METHODS

Materials

Clarithromycin was received as a benevolent gift from from Alkem laboratories Ltd., Mumbai, (India), Eudragit S 100 was also received as gift sample from Deggusa india pvt. Ltd., Mumbai. PVA, dichloromethane, tween 80 was purchased from SD fine chemicals ltd., Mumbai (India). Other chemicals, solvents and reagents were of analytical grade unless otherwise specified.

Preparation and Optimization

Microspheres with a internal hollow structure were prepared by solvent diffusion and evaporation method [11-13]. Accurate quantitiy of polymer i.e. Eudragit S 100 (100 mg) was dissolved in 8ml ethanol followed by addition of 8 ml dichloromethane. Weighted quantity of claithromycin (CTM) was homogeneously dispersed in this polymer solution. This solution was slowly introduced into 200ml of polyvinyl alcohol (0.75% w/v PVA solution) aqueous solution with stirring at 350-400 rpm using a mechanical stirrer (Remi India) equipped with a blade propeller. The solution was stirred for 3-4 hrs and microspheres were collected by centrifugation, washed three times with distilled water and dried at room temperature for 24 hrs. Various formulation and process variables i.e. drug and polymer concentration, surfactant concentration, stirring speed and effect of temperature which could affect the preparation and properties of microspheres were identified and studied. The optimization was done on the basis of particle size, drug entrapment efficiency and buoyancy. The various variables were optimized by varying one variable at a time and keeping other variables constant (Tables 1 and 2).

Characterization of Floating Microsphere

Prepared microspheres were characterized for shape and surface morphology, size, percent drug entrapment, percentage buoyancy and *in vitro* drug release.

Shape and surface morphology:

Shape and surface morphology of the formulations were viewed under scanning electron microscope (Zeiss EVO-40 EP, India). The samples for SEM were prepared by lightly sprinkling the microspheres powder on a double adhesive tape, which stuck to an aluminum stub. The stubs were then coated with gold to a thickness of about 300Å using a sputter water (Figure 1).

Particle size and size distribution:

The size of microspheres was determined using a microscope fitted with an ocular micrometer and stage micrometer (Motic Microscope, India) and the mean particle size was calculated by measuring 100 particles with the help of a calibrated ocular micrometer [14]**.**

In vitro buoyancy profile:

Clarithromycin microspheres (equivalent to 100 mg) were dispensed in 900 ml of 0.01 N HCl solution containing 0.02% of tween 80 to simulated gastric fluid in dissolution apparatus (Type II) at 37 ± 2°C. The medium was agitated with a paddle rotating at 100 rpm for 12 h. The floating and the settled portion of microsphere were recovered separately. The microsphere were dried and weighed. Percent Buoyancy of the total mass of the floating microspheres was determined by following formula [5].

$$\% \text{ Buoyancy} = \frac{W_f}{W_f + W_s} \times 100$$

Where, Wf and Ws are the weights of the floating and settled microspheres, respectively. All the tests were carried out in triplicate.

Entrapment Efficiency

Clarithromycin microsphere equivalent to 100 mg of the drug were taken for evaluation. The amount of drug entrapped was estimated by crushing the microsphere and extracting with aliquots of 0.1 N HCl repeatedly. The extract was transferred to 100 ml volumetric flask and the volume was made up using 0.1 N HCl. The solution was filtered and the absorbance was measured at 287 nm against blank. The amount of drug entrapped in the microsphere was calculated by following formula:

$$\% \text{ Entrapment Efficiency} = \frac{\text{Amount of drug actually present}}{\text{Theoretical drug load expected}} \times 100$$

***In vitro* Drug Release Study**

All formulations of microspheres were evaluated for the *in vitro* drug release study. Drug release from the microspheres was carried out using a USP II paddle dissolution apparatus. Microspheres, equivalent to 50 mg of the drug was placed in packed. The release was tested in dissolution medium of pH 1.2 SGF and pH 7.4 PBS solutions at 100 rpm and thermostatically controlled at 37 ± 0.5°C. Perfect sink condition was prevailed during the drug dissolution. An aliquot of the release medium was withdrawn at predetermined time intervals and an equivalent amount of fresh medium was added to the release medium. The collected samples were filtered through 0.45 µm-syringe filter (Millipore millex HN) and analyzed spectrophotometrically (Figures 2 and 3).

Stability Study

The prepared formulation was studied for stability profile at normal and accelerated conditions as per ICH Q1A (R2) guidelines. The formulation was placed separately in amber colored borosilicate screw capped glass container and stored at normal room temperature 25°C ± 2°C/60% RH ± 5% , freezing temperature 4°C ± 2°C /65 RH) ±5% and for accelerated testing at oven temperature 40°C ± 2°C/75% RH ± 5% RH respectively for a period of 45 days [15]**.** After every 15 days the stored formulations were evaluated for various parameters. Change in color was visualized and size of the formulation was determined by optical microscopy using an ocular micrometer. For the determination of residual drug, microspheres were dissolved in PBS pH 7.4, and then the drug content estimated spectrophotometrically using UV-visible spectrophotometer.

***In vivo* Radiographic Studies**

In order to assess the gastroretentive efficacy of floating formulations, the % buoyancy in a biological system was determined by using 5% w/v barium sulphate X-ray contrast medium act as a contrast agent were prepared for radio graphical study. The optimized formulations which showed good *in vitro* buoyancy were finally selected for radiography study. The drug in all selected formulations was replaced with the same amount of barium sulphate while all other ingredients were kept constant [16]. Prepared formulations were analyzed for their physical properties and it confirmed that the developed dosage forms were similar to those containing drug.

Animal Selected

Hamster Rabbit (male), weighing 2.0-2.5 kg were used for radiographical study of formulation. Then the rabbits placed in cages for two days in quarantine area and kept in standard environmental conditions, fed with standard diet and allowed free access to drinking water. Animals shall be housed in polypropylene cages maintained under standard conditions of 12-h light/dark cycle, 23 ± 2°C and 35-60% humidity. All animals fasted 12 hrs prior to experimental use.

EXPERIMENTAL PROCEDURE

The rabbits are grouped into two; each group consists of six animals. The group-I were treated as conventional group, administered with marketed formulation whereas group II treated with prepared formulation i.e. floating clarithromycin microsphere for once. After 0 hr, 1 hr, 3 hr, and 6 hr the intragastric behavior of the microspheres observed by X- ray. Then the intragastric behaviour was compared with the marketed formulation. In each experiment, the first radiographic image of the animal subjects was taken to ensure the absence of radio-opaque material in the gastrointestinal tract. One of each dosage form (capsule containing microspheres) prepared for radiography was orally administered to rabbits with sufficient amount of water [17].

RESULTS AND DISCUSSION

Preparation and Optimization of Carrier System

The floating microspheres were prepared by solvent diffusion and evaporation method to create the hollow inner core. The Eudragit S100 solution in ethanol/dichloromethane was sequentially dropped into PVA solution and dispersed in external phase. PVA solution was chosen as the external phase because ethanol/dichloromethane (DCM) mixture as an internal phase is not miscible with PVA solution and the Eudragit S100 is not soluble in it. As the dispersed droplets of Eudragit S100 solution collided with those of PVA solution, they formed an inter polymer complex. The droplets of Eudragit S100 gradually solidified and hardened as ethanol and DCM diffused out of the

internal phase [18]. Polymers were dissolved in an organic solvent and then emulsified with an aqueous drug solution containing surfactant to form oil in water emulsion. After the formation of a stable emulsion, the organic solvent was evaporated by continuous stirring. The solvent removal leads to polymer precipitation at the o/w interface of droplets, forming a cavity, and thus imparted the floating properties in the developed microspheres [19]. Formulation variable polymer and surfactant concentration affect the carrier system and optimized on the basis of particle size, percent entrapment efficiency and percent buoyancy (Tables 1 and 2). Polymer concentration influences as the particle size and entrapment efficiency as the lower polymer concentration resulted in low entrapment efficiency and exceeding the polymer concentration above 100 mg further reduced the entrapment efficiency from 78.33% to 73.29%. The reason behind this might be that increase in polymer concentration increases the viscosity of the internal phase increased leading to lesser space for the drug entrapment & easy drug leach out from the internal phase leading to reduced entrapment efficiency. After optimizing concentration of polymer and surfactant, other variables including stirring speed, stirring time and temperature were also optimized for the final formulation. The results of optimized parameters are summarized in Tables 3 and 4.

Table 1: Formulation variable of floating microsphere (polymer concentration)

Ingredient	Formulation			
	E1	**E2**	**E3**	**E4**
Drug (mg)	100	100	100	100
Eudragit S100	60	80	100	120
Surfactant PVA (% W/V)	0.75	0.75	0.75	0.75
Ethanol: Dichloromethane (mL)	08:08	08:08	08:08	08:08

Table 2: Formulation variable of floating microsphere (surfactant concentration)

Ingredient	Formulation			
	F1	F2	F3	F4
Drug (mg)	100	100	100	100
Eudragit S100	100	100	100	100
Surfactant PVA (% W/V)	0.5	0.75	1	1.25
Ethanol: Dichloromethane (mL)	08:08	08:08	08:08	08:08

Table 3: Particle size, (%) Entrapment efficiency and % Buoyancy of floating Eudragit microsphere

Formulation	Particle size (µm)	Entrapment efficiency (%)	% Buoyancy
E1F	91.45 ± 01.92	54.46 ± 03.12%	81.26 ± 1.23
E2F	128.35 ± 03.31	62.37 ± 02.22%	74.34 ± 2.15
E3F	**142.33 ± 02.21**	**78.33 ± 02.84%**	**95.16 ± 0.82**
E4F	151 ± 02.90	73.29 ± 03.62%	78.62 ± 1.69
E3F1	166.47 ± 02.94	82.54 ± 03.24%	79.23 ± 0.95
E3F2	**142.26 ± 03.47**	**79.22 ± 03.46%**	**93.35 ± 2.45**
E3F3	132.65 ± 02.43	73.86 ± 01.64%	86.54 ± 1.11
E3F4	121.84 ± 03.48	67.76 ± 01.97%	76.28 ± 0.98

Table 4: Values of optimized parameters for formulation

S. No.	Factor	Optimized parameter
1	Drug concentration	100 mg
2	Polymer concentration	100 mg
3	Surfactant concentration	0.75 w/v
4	Stirring speed	400 rpm
5	Stirring time	30ºC

In vitro Drug Release Study

In vitro drug release study performed over the period of 24 hr in pH 1.2 and PBS 7.4 and results are shown in Figures 2 and 3. Results confirmed that floating microspheres resulted in sustained and prolonged release of drug in the SGF fluids. Release obeys the first order kinetic model and the drug release was diffusion controlled Fickian transport.

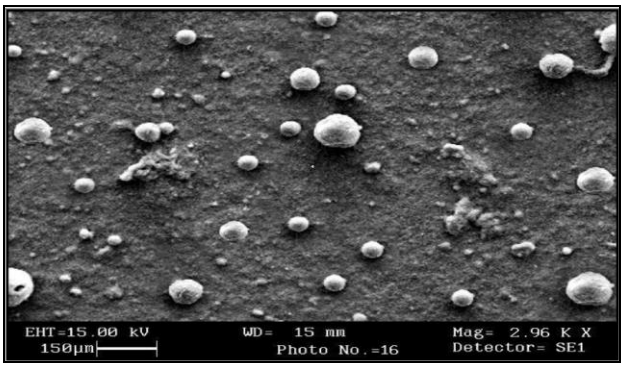

Figure 1: SEM photograph of claritzhromycin microspheres

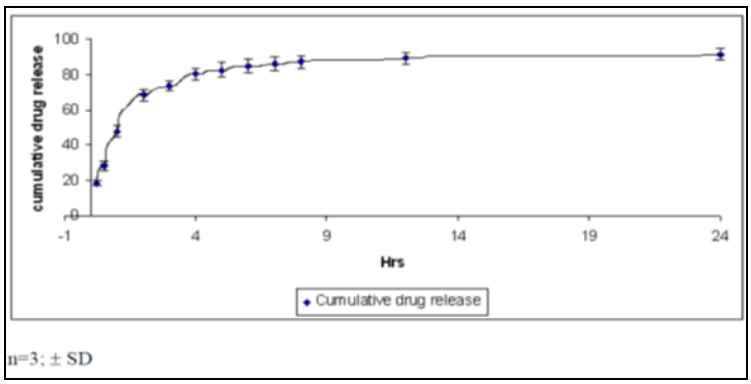

Figure 2: Cumulative % clarithromycin release from Eudragit S100 microspheres in SGF pH 1.2

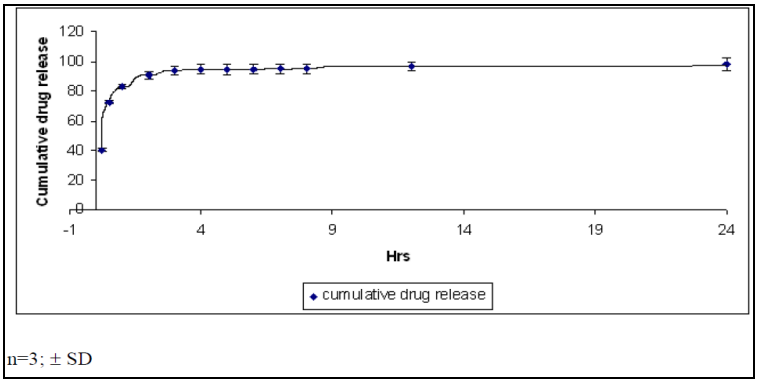

Figure 3: Cumulative % clarithromycin release from Eudragit S100 microspheres at pH 7.4

Stability Study

During storage stability study, particle size of formulation slightly increases after storage of formulation for 45 days. The Little increment in particles size might be due to aggregation of microspheres stored in refrigerated conditions was observed. Drug content of the formulation has no significant variation (Table 5) (Figures 4 and 5).

Table 5: Stability data of optimized formulation stored for 45 days according to the ICH Q1A (R2) guidelines

Observation	Initial observation (0 Days)			Final observation (45 days)		
Factor	4°C ± 2°C/ 65% ± 5% RH)	25°C ± 2°C/ 60% ± 5% RH	40°C ± 2°C/ 75% ± 5% RH	4°C ± 2°C/ 65% ± 5% RH)	25°C ± 2°C/ 60% ± 5% RH	40°C ± 2°C/ 75% ± 5% RH
Particle size	148.23 ± 3.24	148.23 ± 2.42	148.20 ± 3.72	149.2 ± 1.86	149.31 ± 2.23	178.31 ± 2.12
Drug content	100	100	100	98.96 ± 2.13	98.25 ± 2.62	97.87 ± 2.18

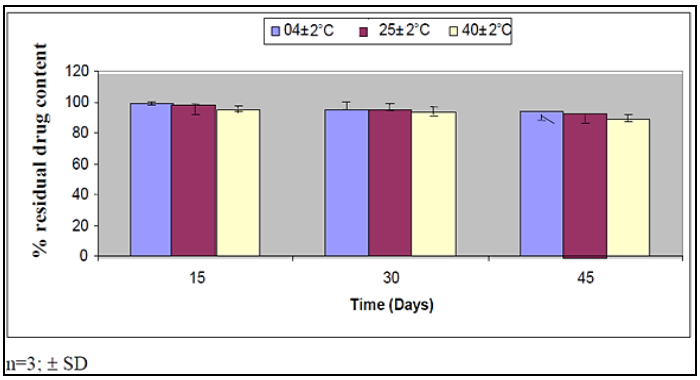

Figure 4: Effect of storage temperature on particle size of microspheres

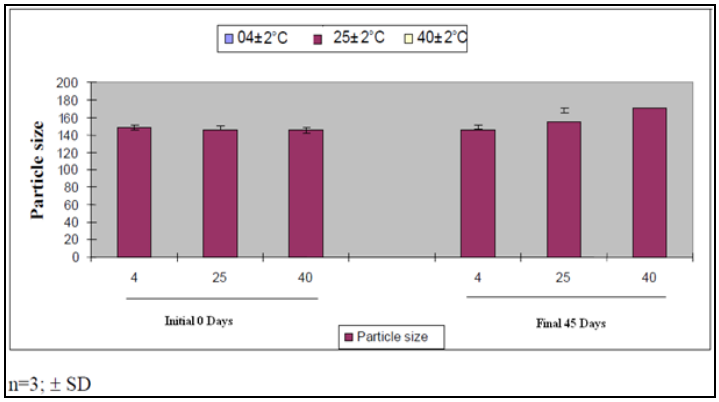

Figure 5: % residual drug concentration in microspheres on storage at different temperatures

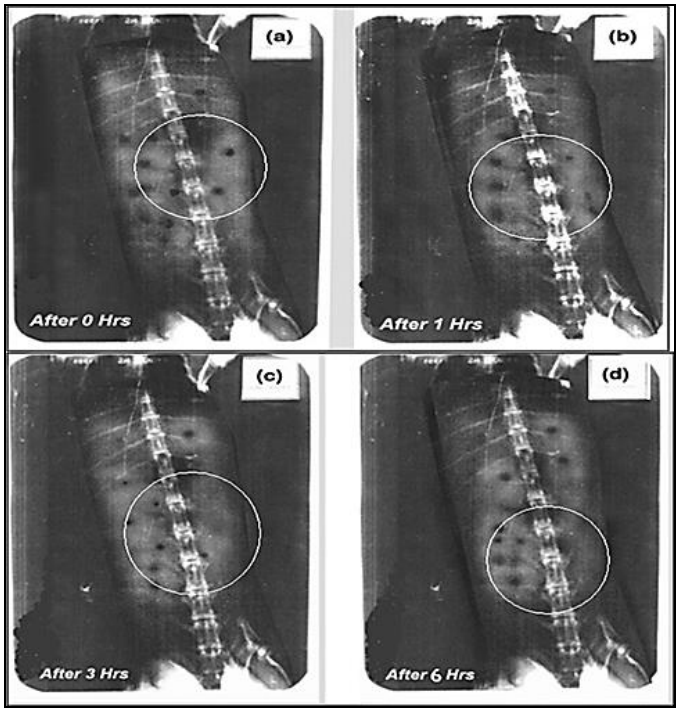

Figure 6: Radiographic images showing the presence of $BaSO_4$ loaded floating microspheres in the stomach at different time intervals. Images were taken after a: 0 h, b: 1 h, c: 3 h, d: 6h

***In vivo* Study**

From *in vivo* radiographical study of floating microspheres entrap BaSo4 as X-ray content we could prove that these microsphere resided in the stomach for a longer period of time, therefore clarithromycin were also reside in the stomach, when it will be administered in the form of floating microspheres (Figure 6).

CONCLUSION

The extreme acidic environment of the stomach, its regular voidance of contents with the antibiotic resistance of the bacteria contributes to the poor success in the treatment of *Helicobacter pylori* gastric infections. Solvent diffusion and evaporation method could effectively be loaded with clarithromycin. The obtained microspheres have particle size 142.26 ± 03.47 nm possessed 79.22 ± 03.46% entrapment efficiency of clarithromycin and 93.35 ± 2.45% buoyancy. From radiographical study of floating microspheres entrap BaSo4 as X-ray content we could prove that these microsphere resided in the stomach for a longer period of time, therefore clarithromycin were also reside in the stomach, when it will be administered in the form of floating microspheres.

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