Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2011, 3 (6):90-101
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0974-248X
USA CODEN: DPLEB4

Formulation and in vitro evaluation of clarithromycin floating microspheres for eradication of Helicobacter Pylori

Bathini Sree Tejaswi*, Durgaramani Sivadasan and Shalini Devi. P

PSG College of Pharmacy, Peelamedu, Coimbatore, Tamilnadu, India

---

ABSTRACT

The objective of the study was to develop a stomach-specific drug delivery system for controlled release of Clarithromycin for eradication of Helicobacter pylori (H. pylori). Floating Microspheres of Clarithromycin (FMC) were prepared by Solvent Evaporation Technique using ethyl cellulose as a polymer. The prepared microspheres were subjected to evaluation for particle size, incorporation efficiency, in vitro buoyancy, in vitro drug release characteristics and stability studies. These microspheres showed good buoyancy. The formulation variables like polymer concentration and drug concentration influenced the in vitro drug release significantly in simulated gastric fluid (pH. 2.0). It was also noted that the required amount of Clarithromycin for eradication of H. pylori was significantly less in FMC than from corresponding Clarithromycin suspension. About 82% of the prepared microspheres floated in hydrochloric acid buffer solution for 12h. 71% of the Clarithromycin contained in the microspheres were released within 12 h in a sustained manner. These results suggest that FMC will be a promising drug delivery system for the treatment of H. pylori infection.

Key Words: Floating Microspheres, Clarithromycin, Ethyl Cellulose, Gastroretentive Drug Delivery.

INTRODUCTION

Floating systems are the low-density systems that have sufficient buoyancy to float over gastric contents and remain in the stomach for a prolonged period. While the system floats over gastric contents, the drug release slowly at the desired rate, which results in the increased gastro-retention time and reduces fluctuation in the plasma drug concentration [8]. A floating dosage unit is useful for the drugs which act locally in the proximal gastrointestinal tract. The drugs which are poorly soluble and unstable in intestinal fluids these systems are useful. The floating properties of floating drug delivery systems help in retaining these systems in stomach for a long time [10]. Various attempts have been made to develop a floating delivery system [7]. This system will float on the gastric contents for desired time period [4]. After the release of drug, the remnants of the system will be emptied from the stomach.

When microspheres come in contact with gastric fluid, the hydration of gel formers, polymers and polysaccharides occurs to form a colloidal gel barrier which controls the rate of fluid penetration and consequent drug release. As the exterior surface of dosage form dissolves, the gel layer is maintained by the hydration of the adjacent hydrocolloid layer [15]. The air entrapped by the swollen polymer lowers the density and imparts buoyancy to the microspheres. However a minimal gastric content is needed to allow proper achievement of the buoyancy [14]. Hollow microspheres of eudragit, acrylic resins, polyethylene oxide, and cellulose acetate; polycarbonate floating balloons and gelucire floating granules; polystyrene floatable shells are the recent developments.

H. pylorus is a Gram-negative, spiral, microaerophilic and flagellated bacterium, with unipolar-sheathed flagella which provides motility [11]. Its spiral shape and high motility allows it to penetrate the mucus, resist gastric emptying and remain in host gastrointestinal (GI) tract. It causes a chronic inflammation of stomach lining and may cause development of the duodenal and gastric ulcers and stomach cancer [13]. H. Pylori weaken the protective mucous coating of duodenum and stomach and allows acid to get through the sensitive lining beneath. Both the acid and bacteria irritate the lining and causes an ulcer. H. *Pylori* are able to survive in the stomach acid as it secretes enzymes which neutralize the acid [3].

Clarithromycin is orally absorbed, macrolide, broad-spectrum antibiotic which is widely used in the standard eradication treatment of *H. Pylori* infection combined with a second antibiotic and an acid-suppressing agent. Clarithromycin has the highest rate of eradication of *H. pylori* in monotherapy *in vivo* and hence was selected as a model drug in this study [5].

In order to improve the efficacy of anti- *H. pylori* clarithromycin, we propose a new concept based on floating system with site-specific drug delivery of clarithromycin using ethyl cellulose as polymer and calcium carbonate as gas forming agent. This stomach-specific delivery system will increase the gastric residence time, decreases the diffusional distance, and allow more of the antibiotic to penetrate through the gastric mucus layer and act locally at the infectious site and also minimize the resistance problems associated with systemic administration of antibiotics.

MATERIALS AND METHODS

2.1. Materials

Clarithromycin was a generous gift from Orchid Pharma (Chennai), Ethyl Cellulose (Low Viscosity) and Carbopol 934P was obtained from Loba Chemie (Mumbai), Calcium Carbonate and Liquid Paraffin was obtained from Qualigens (Mumbai), Hydrochloric Acid obtained from Qualigens, Mumbai.

1.2.Preparation of Floating Microspheres of Clarithromycin

The technique of floating microspheres preparation is based on emulsification solvent evaporation method in which the polymer ethylcellulose was dissolved in 50ml of acetone at different concentrations with stirring. Carbopol 934P, calcium carbonate and clarithromycin of different concentrations were added to the above polymer solution and the total mixture was blended for 2h [9]. Then this suspension was slowly added to the 200ml light liquid paraffin which containing 2.0% Span 80 and stirred at a rate of 1200 rpm using Remi mechanical stirrer equipped with a three bladed propeller at room temperature for 1h [2]. After 1hr of emulsification, acetone was evaporated gradually with the help of a rotary flash evaporator at 40^0C until the microspheres were formed. The formed microspheres were washed with petroleum ether (40^0 - 60^0 C) and dried at room temperature. Fourteen formulations had been

prepared by this method; the various formulation variables considered for optimisation were shown in the table no 1.

Table No1: Formulation Optimisation of Floating Microspheres of Clarithromycin

Formulation Code	Ethylcellulose (%)	Calcium Carbonate (%)	Carbopol (%)	Clarithromycin (%)
F1	2.5	1.5	1	2.5
F2	2.5	2	1	2.5
F3	2.5	2.5	1	2.5
F4	2.5	3	1	2.5
F5	1.5	2	1	2.5
F6	2	2	1	2.5
F7	3	2	1	2.5
F8	2.5	2	0.5	2.5
F9	2.5	2	2	2.5
F10	2.5	2	3	2.5
F11	1	-	-	1
F12	0.5	-	-	1
F13	2	-	-	1
F14	3	-	-	1

1.3.Determination of Particle Size

The particle size of the prepared microspheres was determined by the Phase contrast microscopy. The samples were dispersed in the liquid paraffin and the sizes of microspheres were observed under microscope.

1.4.Determination of Entrapment Efficiency

Accurately 50 mg of dried microspheres were soaked in 50 ml of distilled water and sonicated using probe sonicator for 10 min. The solution was centrifuged for 1hour and the entrapment efficiency of the drug was determined by measuring the concentration of free drug present in the supernatant [16] after centrifugation at 13,000 rpm for 1hr at 4^0C. The concentration of drug present in the supernatant was determined through UV spectrophotometer (Shimadzu 1650, Japan) at 260nm. The following formula determines the percentage drug entrapment:

$$\%EP = \frac{\text{Amount of drug added initially} - \text{Amount of drug present in the supernatant}}{\text{Amount of drug added initially}} \times 100$$

1.5.In Vitro Buoyancy Studies

The floating microspheres about 100 mg were spread over the surface of the dissolution medium of 900ml simulated gastric fluid (SGF, pH 2.0), which is placed in USP dissolution apparatus II. The medium temperature was maintained at 37 °C and was agitated by paddle at 100 rpm. After agitation the microspheres that floated over the surface of the medium and those that settled down at bottom of the flask were recovered separately and dried [1]. The percentage of floating microsphere was determined by the following equation:

$$\text{Buoyancy \%} = \frac{\text{Weight of microspheres floated on medium}}{\text{Wt of microspheres floated on medium+ Wt of microspheres settled at bottom of flask}} \times 100$$

1.6. Stability Studies

The physical and chemical stability of clarithromycin loaded floating microspheres were evaluated by storing the formulations at humidity controlled oven (40^0C), room temperature (28^0C**),** and at refrigeration temperature (2-8^0C) [6]. Samples were withdrawn at 15^{th} day, 30^{th} day and 60^{th} day and were checked for appearance, entrapment efficiency and buoyancy percentage.

1.7. In vitro Drug Release Studies

The *in-vitro* dissolution studies were carried out by using USP II paddle type dissolution apparatus. Accurately 100mg of clarithromycin floating microspheres was introduced into 900 ml of 0.1 N Hcl (pH 2), used as a dissolution medium, maintained at a temperature of 37°C, and a rotational speed of 100 rpm [12]. Samples were withdrawn at predetermined time intervals of every one hour for twelve hours. The samples were analyzed UV spectrophotometrically at 260 nm to determine the percentage of drug release.

RESULTS AND DISCUSSION

3.1. Formulation of Floating Microspheres of Clarithromycin

Floating microspheres loaded with clarithromycin in their outer polymer shells were prepared by emulsification solvent evaporation method. The reason behind the selection of floating microspheres as a drug carrier is:

1. Enhancement of bioavailability despite first pass effect because fluctuations in the plasma drug concentration is avoided.
2. A desirable plasma drug concentration is maintained by continuous drug release.
3. Buoyancy increases gastric retention time.
4. Drug releases in a controlled manner for prolonged period.
5. Avoidance of gastric irritation, because of sustained release effect.
6. Better therapeutic effect of short half-life drugs can be achieved.

By emulsion solvent evaporation method, different ratios of drug and polymer formulations were prepared for formulation optimization. In order to achieve better buoyancy, encapsulation efficiency and mucoadhesive properties various formulation variables like different concentrations of ethyl cellulose, calcium carbonate, carbopol were optimized in the study by trial and error method.

The floating microspheres were prepared with different concentrations of ethyl cellulose (0.5, 1, 2, 2.5 and 3%) to investigate the influence of EC on encapsulation efficiency.Calcium carbonate of 1.5, 2 and 2.5% concentrations were used to determine its influence on floating behaviour.Carbopol of 0.5, 1, 2 and 3% concentrations used to study its effect on mucoadhesive nature.

The different ratios of formulation variables were selected for optimization of their buoyancy property. The formulations in which ethyl cellulose**:** calcium carbonate ratios of 2.5**:**1.5, 2.5**:**2.0, 1**:**1 and 1.5**:**2.0 are giving the better results. In all the above formulations the drug is kept constant at 2.5%.

The formulations, shown in table no.2, are selected as the best formulations depending upon their buoyancy, encapsulation efficiency and mucoadhesive properties. From the results of all the fourteen formulations, it is confirmed that the change in concentrations of ethyl cellulose, carbopol and calcium carbonate influences the properties of the formulations. The formulation

F4 with drug and polymer 1:1 ratio, without calcium carbonate and carbopol is giving the best result of buoyancy property.

The microspheres, having lower densities (having a hollow core) exhibited buoyancy and are expected to be retained in gastric environment for more than 12 hrs. This may be attributed to a decrease in density of microspheres with an increase in polymer concentration.

Table No. 2: Formulations Showing the Better Result

Formulation Code	Drug (%)	Polymer (%)	Calcium Carbonate (%)	Carbopol (%)
F1	2.5	2.5	1.5	1
F2	2.5	2.5	2.0	1
F3	2.5	2.5	2.5	1
F4	1.0	1.0	-	-
F5	2.5	1.5	2.0	1

3.2 Determination of Particle Size

The floating microspheres of clarithromycin, prepared in this study were well-rounded spheres with the size ranging from 3 µm to 5 µm. The phase contrast microscopic images of prepared microspheres were shown in the figure 1.

3.2. Determination of Entrapment efficiency

The percentage entrapment efficiency of various formulation parameters of the prepared microspheres were shown in table no 3. The entrapment efficiency varied from 48.35±2.65 to 69.21±3.95. The formulation F4 is having high encapsulation efficiency of 69.21% and F5 is having low encapsulation efficiency of 48.35%.

The low encapsulation is because of the less concentration of ethyl cellulose than the drug concentration where the quantity of ethyl cellulose is insufficient to entrap the drug. The high encapsulation efficiency is because of the high concentration of ethyl cellulose where the increase in the ethyl cellulose concentration forms larger microspheres encapsulating more amount of drug.

Table No 3: Percentage Entrapment Efficiency

Formulation Code	(%) Entrapment Efficiency
F1	58.11±1.72
F2	62.81±0.53
F3	54.78±1.56
F4	69.21±3.95
F5	48.35±2.65

Values represents ± SD, n=3

3.3. In Vitro Buoyancy Studies

The floating ability of the prepared formulations F1 to F14 was evaluated in Simulated Gastric Fluid SGF (pH 2.0). The percentage of the microspheres that floated on the dissolution surface medium was evaluated and was shown in table no.4. All formulations floated for more than 12 hrs on the SGF USP. The results will help to improve the bioavailability of the basic drugs like clarithromycin. The release did not show any burst effect or lag time, which is indicative of a homogeneous drug distribution.

Figure 1. Phase Contrast Microscopy Images of Prepared Floating Microspheres

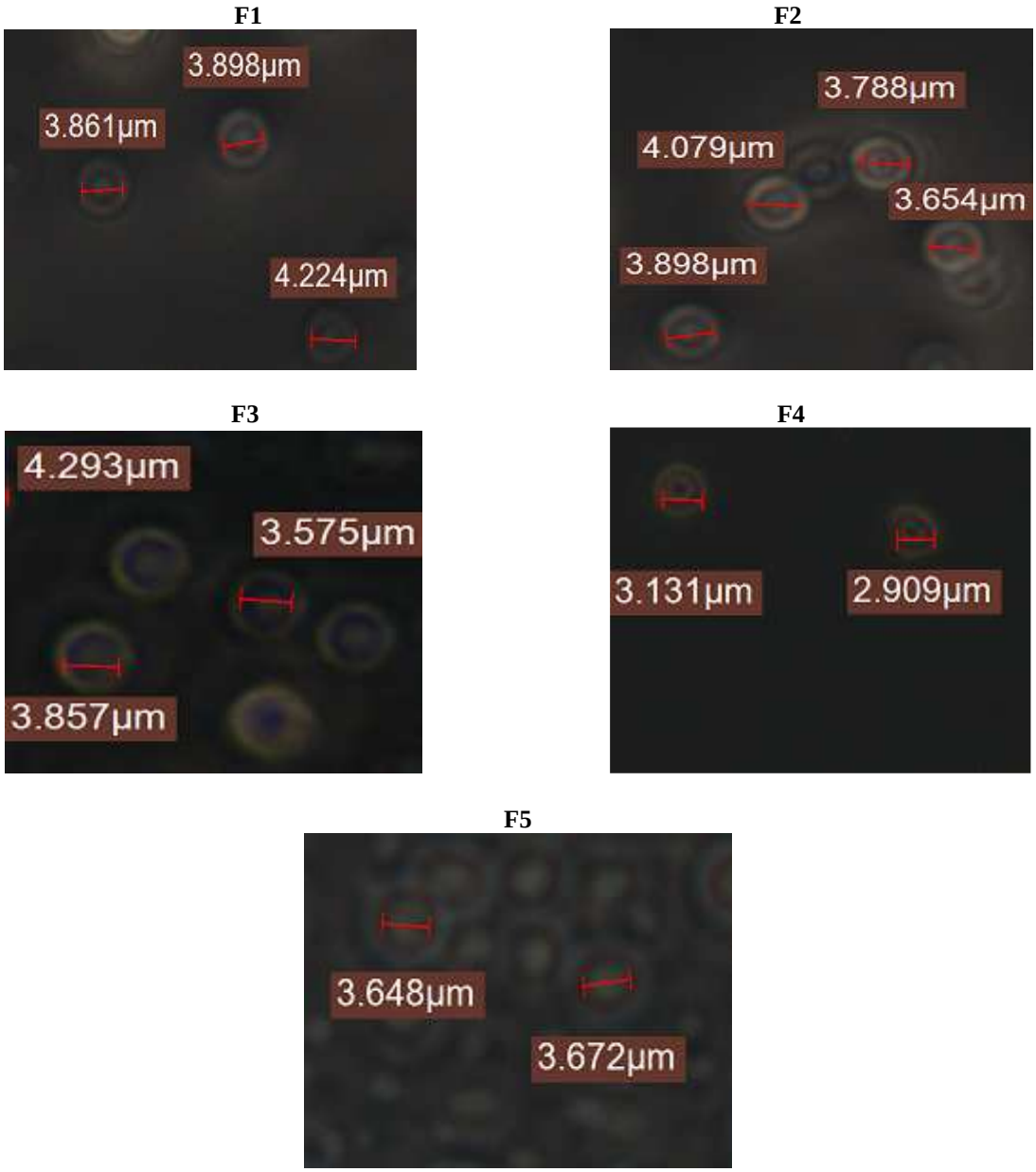


Figure 2. Buoyancy Study of Floating Microspheres

F3

F4

F5

Table No. 4: Percentage Buoyancy

Formulation Code	Buoyancy (%)
F1	66.2±1.75
F2	72.6±1.23
F3	69.6±2.01
F4	86.3±2.45
F5	65.88±3.45
F6	51.3±1.06
F7	53.6±0.79
F8	49.1±1.32
F9	43.4±1.54
F10	45.3±0.78
F11	50.6±2.03
F12	48.9±1.49
F13	40.8±1.04
F14	51.4±0.59

Values represents ±SD, n=3

The formulations which are showing floating percentage more than 65% were selected as the better formulations for prolonging the gastric residence time. The formulations having less ethyl cellulose concentration is having low buoyancy properties because of the increase in the density of microspheres with a decrease in the polymer concentration.

Table No 5: Sampling Time- After 15 Days

Formulation No.	Storage Condition: Room Temp 28^{0}C			Humidity Controlled 40^0 C			Refrigeration Temp 4^0 C		
	Appearance	**(%)EP**	**(%) Buoyancy**	**Appearance**	**(%)EP**	**(%) Buoyancy**	**Appearance**	**(%)EP**	**(%) Buoyancy**
F1	The colour of the microspheres is not changed	57.21%	64.23%	The colour of the microspheres is not changed	56.34%	65.1%	The colour of the microspheres is not changed	58.3%	66.1%
F2		63.32%	73.16%		63.46%	72.3%		64.8%	72.3%
F3		54.93%	69.8%		53.45%	67.2%		52.11%	68.4%
F4		66.31%	86.50%		67.43%	85.4%		68.4%	86.5%
F5		49.1%	64.31%		47.2%	65.0%		48.03%	66.7%

Table No 6: Sampling Time- After 30 Days

Formulation No.	Storage Condition: Room Temp 28^{0}C			Humidity Controlled 40^0 C			Refrigeration Temp 4^0 C		
	Appearance	**(%)EP**	**(%) Buoyancy**	**Appearance**	**(%)EP**	**(%) Buoyancy**	**Appearance**	**(%)EP**	**(%) Buoyancy**
F1	No change in the colour of the microspheres	58.36%	65.4%	No change in the colour of the microspheres	57.21%	65.2%	No change in the colour of the microspheres	58.23%	64.9%
F2		61.3%	73.83%		61.8%	72.1%		63.1 %	71.8%
F3		55.82%	68.7%		53.4%	69.6%		55.01%	68.7%
F4		67.41%	87.5%		68.21%	85.3%		69.41%	85.9%
F5		47.4%	64.8%		47.67%	66.1%		48.5 %	65.12%

Table No 7: Sampling Time - After 60 Days

Formulation No.	Storage Condition								
	Room Temp 28^{0}C			**Humidity Controlled 40^0 C**			**Refrigeration Temp 4^0 C**		
	Appearance	**(%)EP**	**(%) Buoyancy**	**Appearance**	**(%)EP**	**(%) Buoyancy**	**Appearance**	**(%)EP**	**(%) Buoyancy**
F1	No change in the colour of the microspheres	57.9 %	66.8%	No change in the colour of the microspheres	56.36%	65.72%	No change in the colour of the microspheres	58.23%	66.7%
F2		61.1 %	71.6%		61.8 %	72.1%		60.9%	71.56%
F3		54.3 %	69.9%		53.8 %	68.5%		55.3%	68.6%
F4		68.2%	86.8%		67.2 %	86.7%		69.91%	85.9%
F5		48.9%	66.11%		47.13%	64.32%		48.5%	65.8%

Calcium carbonate was added as a gas forming agent but the variations in the concentrations of calcium carbonate is not having a significant effect on buoyancy properties. The formulation F11, F12, F13 and F14 prepared without calcium carbonate are floating for more than 12hrs because of the hollow core formed during the preparation.

3.4. Stability Studies

The stability studies were conducted for two months at three different temperatures of room temperature (28^0C), humidity controlled temperature (40^0C) and refrigeration temperature (4^0C). The sample were analysed for their appearance, % entrapment efficiency and %buoyancy. The results obtained after 15^{th}, 30^{th} and 60^{th} day were shown in the Table no. 5, 6 and 7. The results revealed that there was no change in the appearance. There was a no significant change in the percentage entrapment efficiency and percentage buoyancy after two months of the study when stored at three different temperatures.

3.5. In vitro Drug Release Studies

The *in vitro* drug release studies were conducted in 900 ml 0.1 N Hcl (pH 2.0) simulated gastric fluid as a dissolution medium. The percentage drug release study is done for 12hrs. The *in vitro* drug release profile is shown in the figure 3. The studies revealed that all the formulations are showing the sustained release of the drug. With the increase in the polymer concentration there will be a significant decrease in the rate and extent of drug release.

The formulation F1, F2, F3 and F5 containing ethyl cellulose**:** calcium carbonate ratios 2.5:1.5, 2.5:2.0, 1:1, 1.5:2.0 are having percentage release of 70.5%, 58.7%, 59.9% and 73.5% respectively were shown in table no 8. The formulation F4 with drug:polymer of 1:1 ratio is having 69.7% drug release. The formulation F5 is having highest release of 73.5% than the others, this is because the increase in polymer concentration resulted in better incorporation efficiency of the drug, could be the reason for the observed decrease in drug release since the amount of surface associated drug decreases with an increase in incorporation efficiency.

Table No. 8: *In vitro* Drug Release Profile

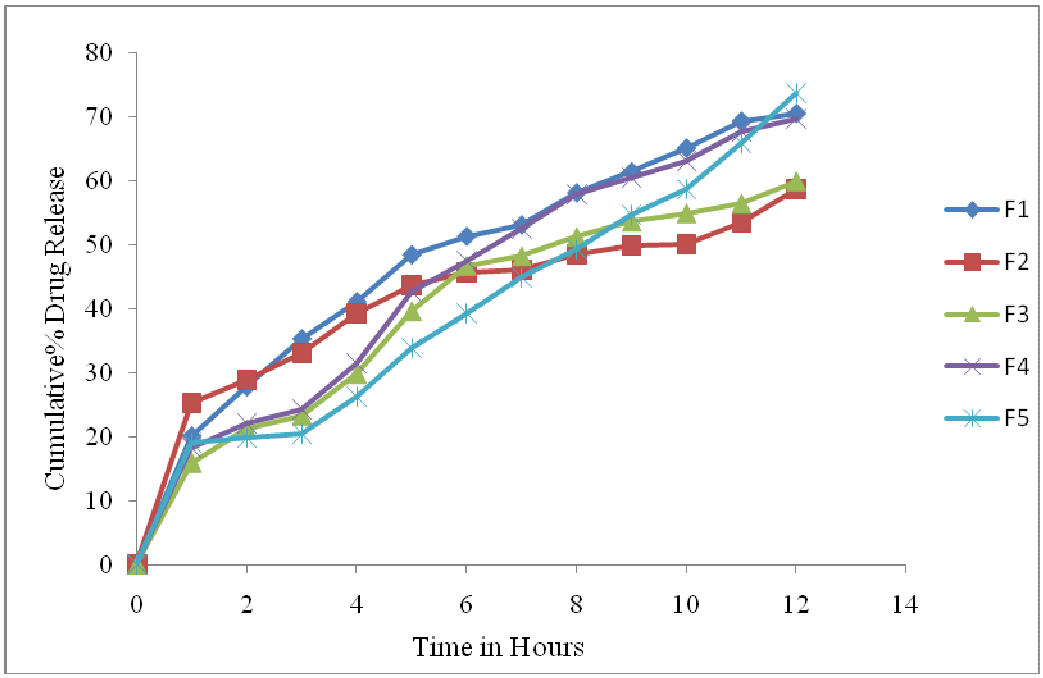
Time in Hours	Cumulative Percentage drug release				
	F1	**F2**	**F3**	**F4**	**F5**
0	0	0	0	0	0
1	20.1±0.6	25.3±0.3	15.9±0.5	18.2±0.9	19.1±1.2
2	27.8±0.9	28.9±0.5	21.2±0.9	22.1±0.2	19.9±0.5
3	35.3±0.4	33.1±0.2	23.3±0.6	24.3±0.5	20.4±0.8
4	41.1±1.1	39.3±0.3	29.8±1.3	31.5±0.1	26.3±0.2
5	48.4±0.6	43.7±0.4	39.6±0.5	42.7±0.6	33.8±1.5
6	51.3±0.5	45.6±0.6	46.7±0.2	47.4±0.4	39.2±0.7
7	53.2±0.3	46.1±1.5	48.2±0.3	52.5±0.1	44.9±0.4
8	58.1±0.2	48.5±0.7	51.2±0.9	57.9±0.8	49.3±1.1
9	61.4±1.9	49.9±0.2	53.2±0.7	60.5±0.2	54.7±0.3
10	65.1±0.5	50.1±0.1	54.9±0.6	63.1±0.6	58.6±0.7
11	69.3±0.2	53.4±0.8	56.4±0.2	67.7±0.4	65.8±0.5
12	70.5±0.7	58.7±0.2	59.9±0.4	69.7±1.3	73.7±0.1

Values represents ±SD, n=3

The variations in calcium carbonate does not having effect on the drug release as it is used as a gas forming agent to prolong the floating of microspheres. An increase in carbopol concentration

caused retardation in drug release from the microspheres because of an increase in the viscosity of polymer solution and formation larger size microspheres.

Figure 3. *In vitro* Drug Release Profile



3.6. In vitro Drug Release Kinetics

Floating microspheres of clarithromycin were prepared by solvent evaporation method using ethyl cellulose to retard release and achieve required release profile. To study the release kinetics, data obtained from *in vitro* release studies were plotted in various kinetic models: Zero order as cumulative amount of drug Vs time, first order as log cumulative percentage of drug released Vs time and Higuchi's model as cumulative percentage of drug released Vs square root of time, Peppas model as log cumulative percent drug released Vs log time.

From the r^2 value it is concluded that the release of drug is of first order. From the peppas model it is indicated that the release is a Non- Fickian diffusion drug release.

CONCLUSION

The ultimate goal for sustained drug release is to maximize therapeutic activity while minimizing the negative side effects of the drug. In this regard, floating microspheres have emerged as a novel drug delivery system for eradication of *Helicobacter pylori* with Clarithromycin.

Clarithromycin was successfully incorporated into the polymer, ethyl cellulose by solvent evaporation method. The obtained microspheres were spherical in shape and the size ranging from 3µm to 5µm. The entrapment efficiency results showed that the drug is encapsulated up to 69%. The buoyancy studies showed that all the formulations were floating more than 12hrs. *In vitro* release profile showed that the drug was released in a sustained manner up to 12hrs. Stabilities studies showed that the formulation can be stored at 40^0C, 28^0C and 4^0C temperatures without affecting drug loading.

From the studies it was concluded that floating microspheres of clarithromycin can be used as stomach specific drug delivery system for the eradication of *H. Pylori* and may provide therapeutic concentration at lower dose of clarithromycin and also may reduce the adverse effects.

Acknowledgement
The authors wish to thank PSG College of Pharmacy for providing all the facilities required to complete this work successfully.

REFERENCES

[1] Anand Kumar Srivastava, Devendra Narayanrao Ridhurkar, Saurabh Wadhwa, *Acta Pharma*, **2005**, 55, 277–285.
[2] Asha Patel, Subhabrata Ray, Ram Sharnagat Thakur, *DARU*, **2006,** 14, 57-64.
[3] Egan, B. J, Katicic, M, Connor, H.J.O, Morain, C. A. O. *Helicobacter*, **2007,** 12, 31–37.
[4] Gupta, GD, Rajeev Garg, *Tropical J. Pharm. Res*, **2010,** 9, 59-66.
[5] Jianhua Zheng, Chaowu Liu, Decai Bao, Yanjun Zhao, Xiaojun Ma. J, *Applied Polymer Science*, **2006,** 102, 2226–2232.
[6] Margret Chandira, Sachin, Debjit Bhowmik, B. Jayakar, *Pharm. Res*, **2009,** 2, 30-42.
[7] Najmuddin, M, Sachin Shelar, Asgar Ali, Patel, V, Khan, T, *Int. J. Applied Pharm*, **2010,** 2, 13-17.
[8] Navneet Syan, Pooja Mathur, Kamal Saroha, Surender Verma, Vipin Kumar, *Arch. Apll. Sci. Res*, **2010,** 2, 257-270.
[9] Paruvathanahalli Siddalingam Rajinikanth, Lakshmi Narayanan Karunagaran, Jagdish Balasubramaniam, C, Brahmeshwar Mishra, *Chem. Pharm. Bull*, **2008,** 56, 1658—1664.
[10] Ramesh,R. Putheti, Mahesh,.C.Patil, *J. Sci & Tec*, **2009,** 4, 1- 12.
[11] Sanjay, S. Patel, Ray, S, Thakur, S. R, *Acta Poloniae Pharmaceutica and Drug Research*, **2006,** 63, 53-61.
[12] Shashikant, D. Barhate, Madhabhai, M. Patel, Amol, B. Patil, Sandeep, R. Pawar, Sumit, R. Rathi, *J. Pharm. Res*, **2009,** 2, 445-448.
[13] Shreeraj, H. Shah, Patel, JK, Patel, NV, *J Chin Integr Med*, **2009,** 7, 976-982.
[14] Shweta Arora, Javed Ali, Alka Ahuja, Roop K. Khar, Sanjula Baboota, *AAPS Pharm. Sci. Tec*, **2005,** 6, 73-90.
[15] Swapnila, D.Vanshiv, Hemant, P. Joshi, Atul, P Sherje, Sanjivani, A.A.phale, Shalaka P Dhat. G, *J. Pharm. Res*, **2009,** 2, 1879-1885.
[16] Venkateswaramurthy, N. Sambathkumar, R. Vijayabaskaran, M, Perumal, P, *Int. J. Current Pharm. Res*, **2010,** 2, 24-27.
[17] Vijaya Raghavan, C. Judith Justin, **2006**, 1, 19.
[18] Vyas, S.P, Khar, R.K, **2004**, 1, 28-29.
[19] Rajeev Garg, Gupta GD, *Tropical Journal of Pharmaceutical Research*, **2010,** 9, 59-66.
[20] Pare, A. Yadav, SK and Patil, UK, *Research J. Pharm. and Tech.*, **2008,** 1, 526-530.
[21] Jayvadan, K. Patel, Madhabhai, M. Patel, *Current Drug Delivery*, **2007**, 4, 41-50.
[22] Deepa, M.K, Karthikeyan, M, *Iranian Journal of Pharmaceutical Sciences*, **2009,** 5, 69-72.