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DEVELOPMENT AND *IN-VITRO* EVALUATION OF FLOATING MICROSPHERES OF CLARITHROMYCIN USING RATE CONTROLLING POLYMER MONTEIRO SS, KAMATH SS, NAYAK S, PRABHU SC, SHABARAYA AR

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Abstract: Gastroretentive systems have the unique quality to remain in the gastric region for several hours. Due to this they prolong the gastric residence time of the drug significantly. Floating microspheres possess the advantage of better flowing properties attributed to the use of low density polymers. Clarithromycin is a broad-spectrum antibiotic and extensively absorbed orally. It is used in the eradication of *H. Pylori* infection combined with an acid suppressing agent. Clarithromycin floating microspheres were prepared using polymer HPMC K15M in different concentrations by solvent evaporation method. The FTIR studies showed no interaction between drug and polymers. The floating microspheres were evaluated for angle of repose, percentage yield, particle size, SEM, buoyancy percentage, drug content, percentage drug entrapment, *in-vitro* dissolution studies, kinetics of drug release and stability studies. Formulation F3 was found to be the best formulation showing the highest degree of sustained release that is 79.59% at the end of 12 hours. Formulations were seen to follow zero order release profile and Korsmeyer-Peppas model was the best fitting model. Marginal changes were observed in the drug content, buoyancy time and *In-vitro* dissolution studies which are insignificant. Storage conditions were carried out at $40\pm 2^{\circ}\text{C}/75\pm 5\%\text{RH}$ for 6 months.

Keywords: Gastroretentive, Clarithromycin, *H. Pylori*, Korsmeyer-Peppas

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INTRODUCTION

The oral route of administration is the most preferred route for the administration of therapeutic agents¹. Conventional dosage forms do not reside in the stomach for prolonged period of time. For dosage forms which reside in the stomach for a prolonged period of time, gastric emptying is an extremely variable process and the ability to control and prolong the emptying time is a valuable asset for such dosage forms.

Gastric residence time of drugs can be significantly prolonged by the use of gastroretentive systems which can remain in the gastric region for several hours. Prolonged gastric retention reduces drug waste, improves bioavailability and improves solubility for drugs that are less soluble in a high pH environment².

Gastric fluids have a bulk density more than that of floating drug delivery systems and hence they remain floating in the stomach without affecting gastric emptying rate for a prolonged period of time. The drug is released slowly at the desired rate from the system while the system remains floating on the gastric contents. The residual system is emptied from the stomach after release of drug. This causes a better control of the fluctuations in plasma drug concentration and an increased gastroretentive time.³

The mechanism of release of drug from microspheres can take place by diffusion, erosion or osmosis.⁴ Floating drug delivery systems have several advantages. For drugs meant for local action on stomach, like antacids. For drugs that are absorbed through the stomach. They are advantageous in case of vigorous intestinal movement and in diarrhoea in order to keep the drug in floating condition in stomach to get a better response.⁵

Helicobacter Pylori is a bacterium commonly found in the stomach. It is capable of causing a number of digestive problems including ulcers and stomach cancer, although much less commonly. Peptic ulcers are caused by the bacterium by damaging the mucous coating that protects the stomach and duodenum. *H. Pylori* and the stomach acid together irritate the lining of the stomach and duodenum and cause an ulcer.

Antibiotics are used to kill *H. Pylori*. Clarithromycin is a macrolide, broad spectrum antibiotic that is orally absorbed. In combination with a second antibiotic and an acid suppressing agent, Clarithromycin is used in the standard eradication of *H. Pylori* infection. It is seen to have the highest rate of eradication of *H. Pylori* in monotherapy *in-vivo*.^{6,7,8,9}

Clarithromycin exhibits its mechanism of action by penetrating bacterial cell wall and reversibly binding to domain V of the 23S ribosomal RNA of the 50S subunit of the bacterial ribosome, hence blocking translocation of aminoacyl transfer-RNA and polypeptide synthesis.^{10,11,12}

In the present study, different concentrations of rate controlling polymer HPMC K15M were used in order to get the best controlled release formulation using appropriate concentration of polymer.

MATERIALS AND METHODS

Clarithromycin, Dichloromethane and HPMC K15M were purchased from Yarrow chemical, Mumbai, India. Ethanol, Tween 80 and Hydrochloric acid were purchased from Loba chemicals, Mumbai, India. Light liquid paraffin purchased from Hi- media, Mumbai, India. Coconut oil from Subhamangala oil mill, Udupi, India.

Spectrum measurement

A solution of Clarithromycin containing the concentration 20µg/ ml was prepared in 0.1N HCL and UV spectrum was taken using Jasco V-630 UV spectrophotometer. The solution was scanned in the range of 200 – 400 nm.

Construction of calibration curve of Clarithromycin in 0.1N HCl

25mg of Clarithromycin was accurately weighed and transferred into a 25ml volumetric flask and dissolved in small quantity of 0.1N HCl. The volume was made up to 25ml with 0.1N HCl to get a solution concentration of 1000µg/ml as stock solution A. 10 ml of stock solution A was again diluted to 100 ml with 0.1N HCl to get a stock solution B of 100 µg/ml. Then further dilutions were made with same medium to get the solution in ranging from 4µg/ml to 20µg/ml.

Drug polymer interaction (FTIR) study

FTIR spectroscopy was performed on Fourier transformed infrared spectrophotometer. The pellets of drug and potassium bromide were prepared by compressing the powders at 20 psi for 10 min on KBr-press and the spectra were scanned in the wave number range of 4000- 600 cm⁻¹. FTIR study was carried on Clarithromycin, individual polymers, physical mixture of Clarithromycin and polymers, Clarithromycin loaded microspheres.

Table 1: Formulation design for Clarithromycin floating microspheres

Ingredients	F1	F2	F3
Clarithromycin	250mg	250mg	250mg
HPMC K15M	250mg	500mg	750mg
Ethanol	20ml	20ml	20ml
Dichloromethane	20ml	20ml	20ml
Light liquid paraffin	50ml	50ml	50ml

Coconut oil	50ml	50ml	50ml
Tween 80	0.3ml	0.3ml	0.3ml

Preparation of Clarithromycin floating microspheres¹³

Method used: Solvent evaporation method

Floating microspheres were prepared by solvent evaporation technique. Accurately weighed drug and HPMC K15M were dissolved in ethanol and dichloromethane (1:1) to form a homogenous polymer solution. This solution is poured dropwise with needle in light liquid paraffin and coconut oil (1:1) containing few drops of tween 80. This is maintained at 30-40°C subsequently stirred at agitation speed of 1000rpm for 4hrs to allow the volatile liquid to evaporate. The microspheres formed were filtered, washed with petroleum ether and dried in vacuum. The microspheres were then stored in a desiccator over fused calcium chloride.

EVALUATION PARAMETERS

Angle of repose¹⁴

The angle of repose of hollow microspheres was determined by fixed funnel method. The hollow microspheres were allowed to fall freely through a funnel until apex of conical pile just touched the tip of the funnel. The angle of repose θ was determined according to the following formula:

$$\theta = \tan^{-1} h / r$$

Where, h = height of pile, r = radius of the pile formed by the hollow microspheres.

Percentage yield¹⁵

The microspheres were dried and weighed to a constant weight in a desiccator and this was noted as the practical yield. The percentage yield of prepared Clarithromycin floating microspheres was determined by using the formula.

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

Particle size¹⁶

Determination of average particle size of Clarithromycin floating microspheres was carried out by optical microscopy in which a calibrated stage micrometer was employed. A minute quantity

of Clarithromycin floating microspheres was spread on a clean glass slide and average size of 300 Clarithromycin floating microspheres was determined in each batch.

Scanning electron microscopy (S.E.M)^{14, 15, 16}

SEM has been used to determine particle size distribution, surface topography, texture, and to examine the morphology of fractured or sectioned surface. Dry Clarithromycin floating microspheres were placed on an electron microscope brass stub and coated with in an ion sputter. Picture of Clarithromycin floating microspheres were taken by random scanning of the stub. Microphotographs were taken on different magnification and higher magnification (500x) was used for surface morphology. SEM studies were carried out by using JEOL JSM-6380LA scanning microscope (Japan).

Buoyancy percentage^{14,15}

50mg of the floating microspheres were placed in 0.1N HCL, 100 ml containing 0.02% w/v span 80. The mixture was stirred at 100 rpm in a magnetic stirrer. After 12 hrs, the layer of buoyant microspheres was pipette and separated by filtration. Particles in the sinking particulate layer were separated by filtration. Particles of both types were dried in a desiccator until constant weight. Both the fractions of microspheres were weighed and buoyancy was determined by the weight ratio of floating particles to the sum of floating and sinking particles.

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

Where W_f and W_s are the weights of the floating and settled microspheres, resp.

Drug content^{15,17}

Practical drug content was analyzed by using the following procedure, weighed amount of Clarithromycin floating microspheres equivalent to 20 mg of Clarithromycin floating microspheres was dissolved in 100 ml of 0.1 M HCl. This solution was kept overnight for the complete dissolution of the Clarithromycin floating microsphere in 0.1M HCl. This solution was filtered and further diluted to make a concentration of 10 µg/ml solution. The absorbance of the solutions was measured at 278nm using double beam UV-Visible spectrophotometer against 0.1M HCl solution as blank and calculated for the percentage of drug present in the sample.

Determination of percentage drug entrapment (PDE)¹⁷

Efficiency of drug entrapment for each batch was calculated in terms of percentage drug entrapment as per the following formula.

$$\text{PDE} = \frac{\text{Practical drug content}}{\text{theoretical drug content}} \times 100$$

***In vitro* dissolution studies^{15,18}**

The release rate of Clarithromycin floating microspheres was determined by employing USP I apparatus by rotating basket method. The dissolution test was performed using 900 ml 0.1N HCl, in $37 \pm 0.5^\circ\text{C}$ at 50 rpm. Clarithromycin floating microspheres equivalent to 250 mg of Clarithromycin were placed in a basket to avoid floating of microspheres. A sample (5 ml) of the solution was withdrawn from the dissolution apparatus hourly for 12 hrs, and the samples were replaced with fresh dissolution medium. The samples were passed through Whatman filter paper and the absorbance of these solutions was measured at 278nm. Dissolution profiles of the formulations were analyzed by plotting drug release versus time plot. Data obtained was also subjected to kinetic treatment to understand release mechanism.

Kinetics of drug release^{15,19}

To examine the drug release kinetics and mechanism, the cumulative release data were fitted to models representing zero order (Q v/s t), first order [$\text{Log}(Q_0-Q)$ v/s t], Higuchi's square root of time (Q v/s $t^{1/2}$) and Korsmeyer Peppas double log plot ($\text{log } Q$ v/s $\text{log } t$) respectively, where Q is the cumulative percentage of drug released at time t and (Q_0-Q) is the cumulative percentage of drug remaining after time t.

In short, the results obtained from *in vitro* release studies were plotted in four kinetics models of data treatment as follows.

- Cumulative percentage drug release Vs. Time (zero order rate kinetics)
- Log cumulative percentage drug retained Vs. Time (first order rate kinetics)
- Cumulative percentage drug release Vs. \sqrt{t} (Higuchi's classical diffusion equation)
- Log of cumulative percentage drug release Vs. log Time (Peppas exponential equation)

If n value is 0.45 or less, the release mechanism follows "Fickian diffusion" and higher values of 0.45 to 0.89 for mass transfer follow a non-fickian model (anomalous transport). The drug release follows zero-order drug release and case II transport if the n value is 0.89. For the values of n higher than 0.89, the mechanism of drug release is regarded as super case II transport. The model is used to analyze the release of pharmaceutical polymeric dosage forms when the release mechanism is not known or more than one type of release phenomenon was involved. The n value could be obtained from slope of the plot of log cumulative % of drug released Vs log time.

Stability studies²⁰

From the prepared floating microspheres which showed appropriate balance between the buoyancy and the percentage release was selected for stability studies. The prepared formulation (F3) were placed in borosilicate screw capped glass containers and stored at temperature (40 ± 2 °C and 75% ± 5% RH) for a period of 180 days. The samples were tested for drug content, buoyancy, percentage cumulative drug released.

RESULTS AND DISCUSSIONS

The λ maximum was found at 278 nm. The standard graph was obtained between range of 0 to 20µg/ml and was found to be linear with regression value of 0.999, shown in figure 1.

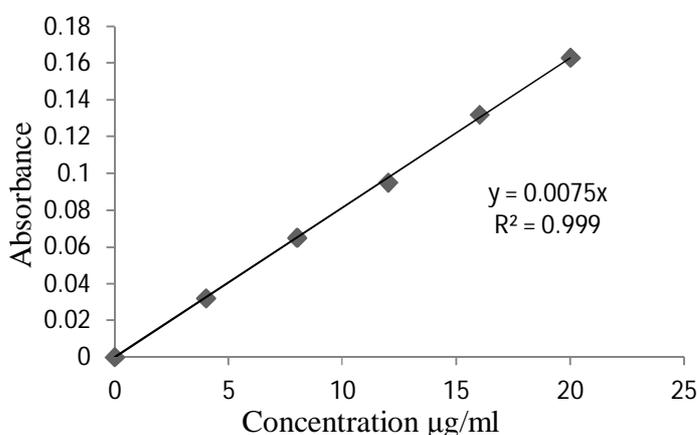


Figure 1: Calibration curve of Clarithromycin

The FTIR studies revealed that characteristics peaks found in Clarithromycin also appeared in physical mixture of drug and polymer, hence it appears there was no chemical interaction between Clarithromycin and polymer and it can be concluded that the characteristics bands of Clarithromycin were not affected after successful loading.

Angle of repose:

Table 2: Results for angle of repose

Sr. No.	F1	F2	F3
Angle of repose	26 ⁰ .56'	23 ⁰ .74'	20 ⁰ .55'

The formulations with HPMC K15M show angle of repose value in the range of 20⁰.55' to 26⁰.56' i.e., less than 30, which shows good flow properties of the formulation F1 and excellent flow property for formulations F2 and F3.

Percentage yield and particle size:

Table 3: Results for percentage yield and particle size

Sr. No	Formulation code	Percentage Yield	Average particle size (µm)
1	F1	70.42%	75.42
2	F2	81.80%	77.27
3	F3	85.31%	80.52

The percentage yield for Clarithromycin floating microspheres for formulation F1, F2, F3 i.e. with HPMC K15M were 70.42%, 81.80% and 85.31% respectively.

It was observed that as the drug to polymer ratio was increased, the mean particle size of Clarithromycin floating microspheres was also increased. The significant increase may be because of the increase in the viscosity of the droplets which may be attributed due to the increase in concentration of the polymer solution. It was observed that clarithromycin floating microspheres with HPMC K15M have average size range of 75.42 µm to 80.52 µm.

Surface morphology of Clarithromycin microspheres (SEM)

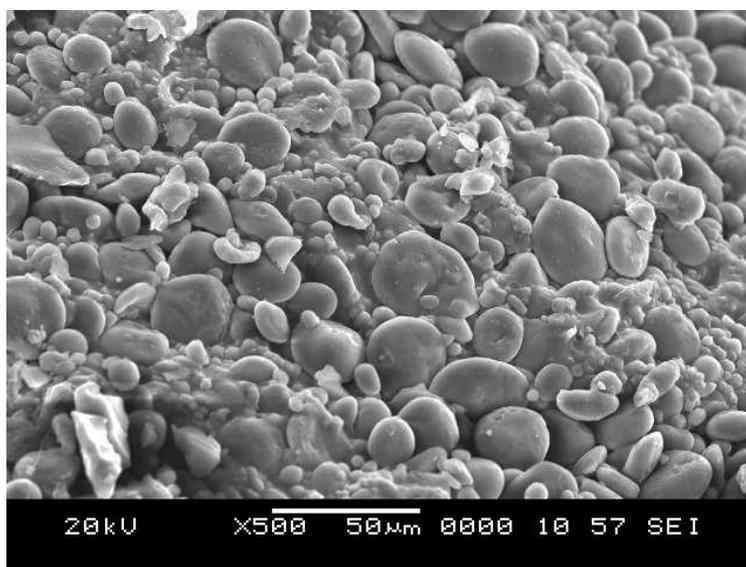


Figure 2: SEM photograph of Clarithromycin floating microspheres

The SEM of microspheres shows a hollow spherical structure with a smooth surface morphology and exhibited a range of sizes within each batch. The floating microspheres were found to be white or almost white in color. Some of the microspheres showed a dented surface structure but they showed good floating ability on the surface of the medium, indicating intact

surface. The outer surface of the microspheres was smooth and dense, while the internal surface was porous. The shell of the microspheres also showed some porous structure. It may be caused by the evaporation of solvent entrapped within the shell of microspheres after forming a smooth and dense skin layer.

Buoyancy Percentage:

Table 4: Results for buoyancy percentage of Clarithromycin floating microspheres

Sr. No.	Formulation code	% Buoyancy time
1	F1	67.31±0.71
2	F2	75.36±0.76
3	F3	80.24±0.61

Values expressed in mean ± standard deviation (n=3)

The microspheres floated for prolonged time over the surface of the dissolution medium without any apparent gelation. This is due to the porosity, apparent density and nature of the polymer. As the polymer concentration increases the buoyancy time increases.

Percentage buoyancy of the Clarithromycin floating microspheres with HPMC K15M was in the range 67.31% to 80.24% after 12 hrs.

Drug content and entrapment efficiency

Table 5: Results for drug content and entrapment efficiency of Clarithromycin floating Microspheres

Sr.no	Formulation Code	Drug content (mg)	Entrapment Efficiency (%)
1	F1	16.42±0.68	82.12±0.93
2	F2	16.83±0.56	84.17±0.97
3	F3	17.47±0.53	87.37±0.95

Values expressed in mean ± standard deviation (n=3)

Entrapment efficiency increases with increase in the polymer concentration. From the results it can be inferred that there is a proper distribution of Clarithromycin in the microspheres and the deviation is within the acceptable limits.

The drug content in the formulations with HPMC K15M was found to be in the range of 16.42mg to 17.47mg. The percentage entrapment efficiency for the formulations with HPMC K15M was found to be in the range of 82.12% to 87.37%.

A maximum of 87.37% drug entrapment efficiency was obtained in the Clarithromycin floating microspheres which were prepared by using HPMC K15M. It was further observed that the drug entrapment was proportional to the Clarithromycin : polymer ratio and size of the Clarithromycin floating microspheres. Increased polymer in a fixed volume of organic solvent has been demonstrated to increase drug retention in floating microspheres. By increasing the polymer concentration, the encapsulation efficiency was increased.

In-vitro dissolution studies of Clarithromycin floating microspheres:

Table 6: Percentage cumulative drug release from formulation F1, F2 and F3

Sr.no.	Time (h)	% Cum. drug release		
		F1 ± SD	F2 ± SD	F3 ± SD
1	0	0	0	0
2	1	18.68±0.14	14.14±0.12	11.44±0.36
3	2	23.61±0.23	18.27±0.21	17.25±0.21
4	3	31.46±0.26	27.11±0.26	24.29±0.09
5	4	38.15±0.27	34.79±0.22	32.68±0.31
6	5	43.91±0.36	41.16±0.32	37.06±0.19
7	6	54.43±0.29	49.01±0.27	43.68±0.11
8	7	62.98±0.12	55.55±0.22	48.74±0.17
9	8	74.71±0.19	69.7±0.12	56.68±0.27
10	9	83.12±0.22	75.1±0.17	61.76±0.33
11	10	91.83±0.26	80.52±0.16	65.04±0.37
12	11	94.44±0.09	84.14±0.25	74.46±0.16
13	12	95.09±0.16	89.03±0.32	79.59±0.14

Values expressed in mean ± standard deviation (n=3)

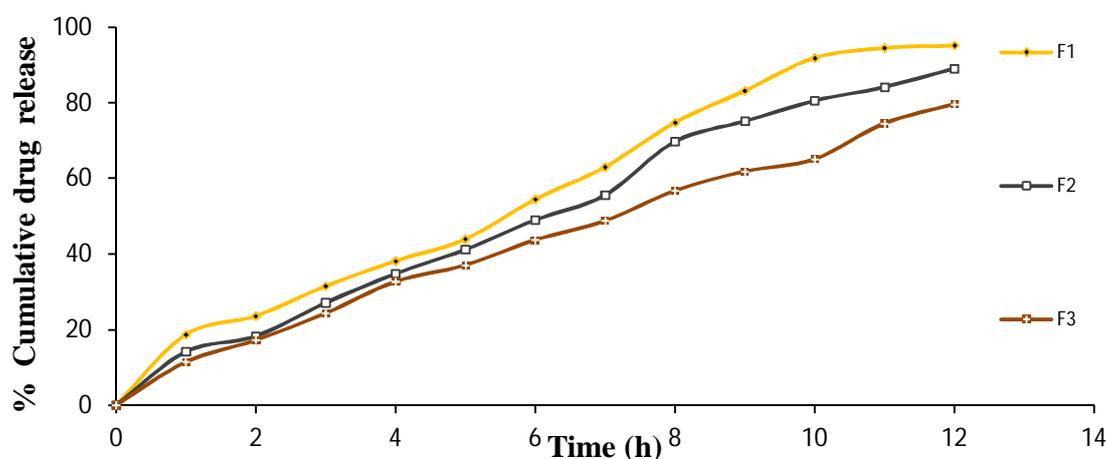


Figure 3: In vitro release profile of formulations F1, F2 and F3

The *in-vitro* performance of Clarithromycin floating microspheres showed prolonged and controlled release of Clarithromycin. The results of the *in-vitro* dissolution studies shows controlled and predictable manner as the polymer concentration increases the drug release from the floating microsphere decreases. This is due to increase in polymer thickness and hence increase in diffusion and erosion pathways. The formulations with HPMC K15M i.e. F1 shows the highest release of 95.09% at the end of twelve hours and F3 shows the least release of 79.59% at the end of twelve hours.

Kinetics of drug release:

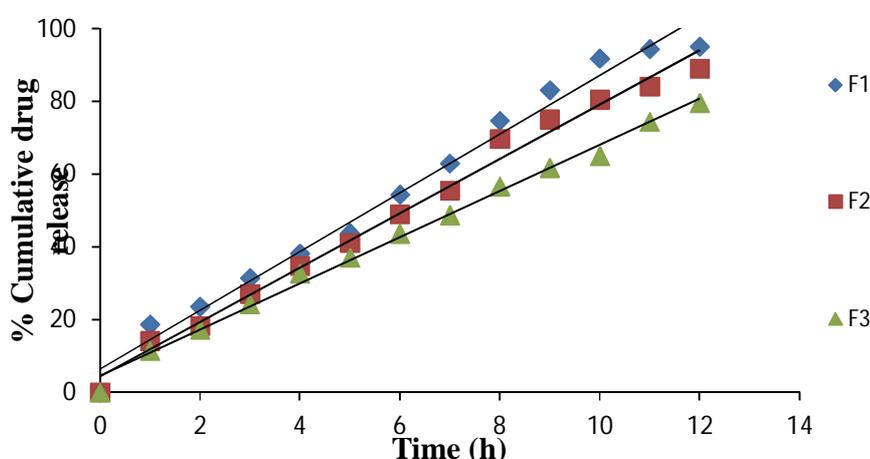


Figure 4: Zero order release kinetics profile of Clarithromycin floating microspheres with HPMC K15M

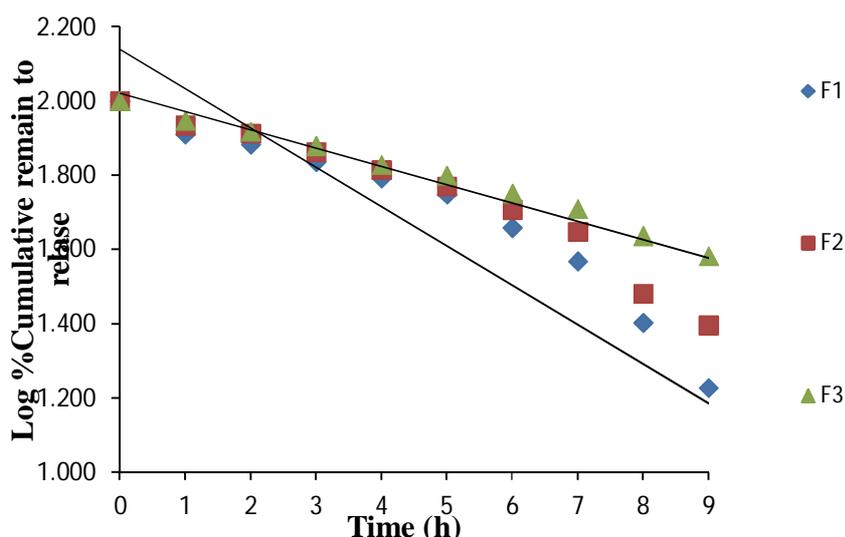


Figure 5: First order release kinetics profile of Clarithromycin floating microspheres with HPMC K15M

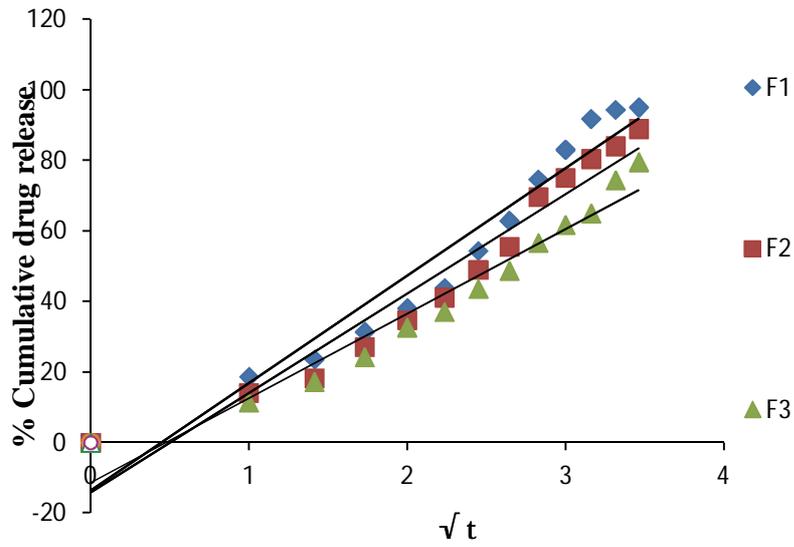


Figure 6: Higuchi matrix diffusion release kinetics profile of Clarithromycin floating microspheres with HPMC K15M

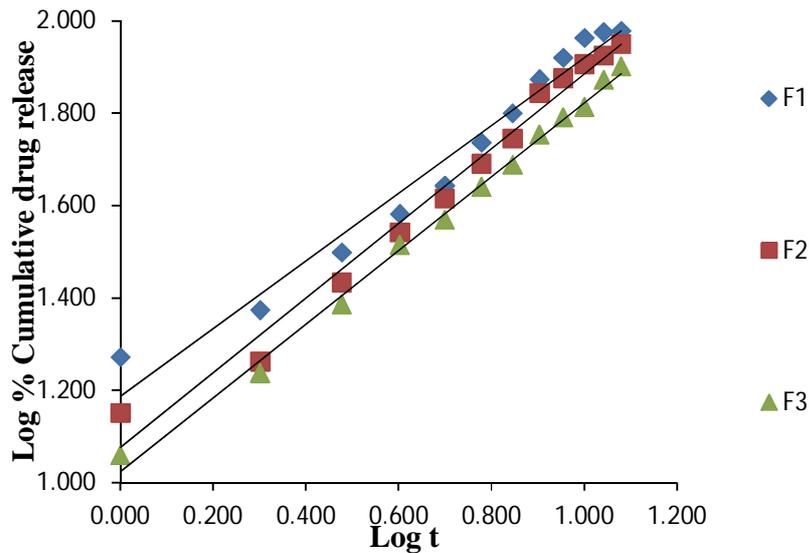


Figure 7 : Peppas model release kinetics profile of Clarithromycin floating microspheres of HPMC K15M

Table 7: Regression co-efficient (r^2) values of different kinetic models and diffusion exponent (n) of Peppas model for Clarithromycin floating microspheres with HPMC K15M.

Formulation	Zero order	First order	Higuchi Matrix	Peppas plot	
				r^2 value	'n' value
F1	0.9843	0.9073	0.9408	0.9697	0.7330
F2	0.9900	0.9533	0.9408	0.9823	0.8084
F3	0.9942	0.9639	0.9507	0.9955	0.7983

To study the release mechanism of Clarithromycin floating microspheres, various dissolution models were applied to the *in-vitro* release profiles of different formulations. These kinetic models include zero order, first order, Higuchi and Korsmeyer-Peppas equations. The plots of Cumulative percentage drug release V/s. Time, Cumulative percent drug retained V/s. root Time, Log Cumulative percent drug retained V/s. Time and Log Cumulative percent drug release V/s. Log Time were drawn and represented graphically.

The slopes and the regression co-efficient of determinations (r^2) are listed in table 7. The co-efficient of determination indicated that the release data was best fitted with zero order kinetics. The diffusion exponent 'n' values of Korsmeyer-Peppas model was found to be in the range of 0.5 to 1 for the Clarithromycin floating microspheres prepared with HPMC K15M indicating Non-Fickian of drug release through Clarithromycin floating microspheres.

Stability studies:

Table 8: Stability results

Time (Days)		% Buoyancy time	Drug Content (%)
		F3	F3
0	–	80.24±0.61	17.47±0.53
90	At 40 ± 2 °C/ 75 ± 5 % RH	79.76±0.41	17.21±0.31
180	At 40 ± 2 °C/ 75 ± 5 % RH	79.43±0.32	17.01±0.29

Table 9: Percentage cumulative drug release from formulation F3, after a period of 0 days, 90 days and 180 days.

Sr.no.	Time (h)	% Cum. drug release		
		0 Days	90 Days	180 Days
1	0	0	0	0
2	1	11.44	11.96	12.47
3	2	17.25	17.7	18.23
4	3	24.29	24.68	25.17
5	4	32.68	33.11	33.77
6	5	37.06	37.62	38.06
7	6	43.68	44.15	44.61
8	7	48.74	49.21	49.78
9	8	56.68	57.17	57.62
10	9	61.76	62.27	62.71
11	10	65.04	65.59	66.03
12	11	74.46	75.01	75.63
13	12	79.59	80.07	80.67

The accelerated stability studies were carried out for the best formulation (F3) at $40\pm 2^{\circ}\text{C}/75\pm 5\%$ RH for six month. The results indicated that the floating microspheres did not show any visual changes (moisture, colour) during the study period and the drug content was found to be 17.01mg at the end of six months. Percentage buoyancy time was found to be 79.43%. There were no significant differences found in the percentage cumulative drug release after stability study. This indicates that the microspheres are fairly stable at storage condition.

CONCLUSION

Floating microspheres of Clarithromycin were prepared successfully by emulsion solvent evaporation using different concentrations of HPMC K15M. As the drug to polymer ratio was increased, the percentage yield of Clarithromycin floating microspheres was also increased. The average particle size of Clarithromycin floating microspheres have increased with an increase in its drug to polymer ratio. By SEM studies, microspheres show a hollow spherical structure with smooth surface morphology and exhibit a range of sizes within each batch. Buoyancy time increases with increase in polymer concentration. Microspheres containing HPMC K15M in the ratio of 1:3 show highest buoyancy percentage. Entrapment efficiency increases with an increase in polymer concentration. Microspheres prepared by using HPMC K15M in F3 showed higher entrapment efficiency. *In-vitro* release studies showed that microspheres containing HPMC K15M in formulation F3 showed a larger degree of sustained release. As the polymer concentration increases the amount of drug released decreases. Drug release kinetic studies

reveal that the release data was best fitted with zero order kinetics. The diffusion exponent n value of Korsmeyer-Peppas model was found to be in the range of 0.5-1 for the Clarithromycin floating microspheres containing HPMC K15M in all three formulations indicating Non-Fickian of drug through Clarithromycin floating microspheres. Best formulation F3 was found to be stable over the specified storage period.

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