

Formulation, Optimization, *in vitro* and *in-vivo* evaluation of levofloxacin hemihydrate Floating Tablets

Muhammad Sohail Arshad¹, Munazza Kiran¹, Jahanzeb Mudassir^{1*},
Muhammad Farhan¹, Amjad Hussain², Nasir Abbas²

¹Department of Pharmaceutics, Faculty of Pharmacy, Bahauddin Zakariya University, Multan, Pakistan, ²College of Pharmacy, University of the Punjab, Lahore, Pakistan

The objective of the present investigation was to design, optimize and characterize the gastro retentive floating levofloxacin tablets and perform *in-vivo* evaluation using radiographic imaging. The floating tablets were prepared by using polymers i.e hydroxy propyl methyl cellulose (HPMC-K4M) and carbopol-940 individually and in combination by nonaqueous granulation method. All the Formulations were evaluated for swelling index (S.I), floating behavior and in-vitro drug release kinetics. The compatibility study of levofloxacin with other polymers was investigated by FTIR, DSC, TGA and XRD. Results from FTIR and DSC revealed no chemical interaction amongst the formulation components. The optimized formulation (F11) showed floating lag time (FLT), total floating time (TFT) swelling index (S.I) of 60 sec, >16h and approximately 75 %, respectively. Moreover, F11 showed zero order levofloxacin release in simulated gastric fluid over the period of 6 h. X-ray studies showed that total buoyancy time was able to delay the gastric emptying of levofloxacin floating tablets in rabbits for more than 4 hours. In conclusion the optimized formulation (F11) can be used for the sustained delivery of levofloxacin for the treatment of peptic ulcer.

Keywords: Gastroretentive dosage forms. Levofloxacin. Floating tablets. Carbopol 940. HPMC K4M.

INTRODUCTION

Peptic ulcer disorder is characterized by painful sores in stomach lining and also affects the first part of small intestine. The causative organism for stomach and duodenal ulcers is *H. Pylori* which survives the acid environment of gastric cavity. Although different antibiotics are effective against *H. Pylori*, successful eradication of this pathogen requires an extended gastric residence time of the antimicrobial agents. Various approaches that have been developed to improve the retention of oral dosage form in the stomach. These include; floating system and non-floating system (Bhardwaj, Sharma, Malviya, 2011). The floating systems have bulk density lower than

that of the gastric fluid and remain floating for longer time. These are further classified as effervescent or non-effervescent depending upon the inclusion or exclusion of gas forming mechanism. The slower drug release rates are achieved by water penetration or diffusion of drug from the floating system. For the reason a minimum floating force is required to keep the dosage form buoyant at the surface of physiologic fluid (Arora *et al.*, 2005).

Non floating system includes bioadhesive system, high density system, expandable system that use different mechanisms to prevent the exit of drugs through pyloric sphincter (Patil, 2015). However, non-floating systems pose severe drawback of sinking in the stomach due to high density.

Floating systems utilize highly swellable and gel forming hydrocolloids. A variety of materials such as hydroxyl propyl methyl cellulose (HPMC), carboxy

*Correspondence: J. Mudassir. Department of Pharmaceutics. Faculty of Pharmacy. Bahauddin Zakariya University. Multan, Pakistan

methyl cellulose and carbopol are used to achieve desired swelling and drug release properties. HPMC takes up water after exposure to aqueous medium and form a gel that control the drug release (Kshirsagar, Jain, Wattamwar, 2009). The viscosity of the mass depends upon the concentration of methoxy group (higher the concentration indicates the more viscosity). The other properties such as prompt hydration, fine compression, easy to use and low toxicity are attractive characteristics to formulation scientist. Carbopol is a high molecular weight, cross linked polyacrylic acid. The polymers are categorized on the basis of cross linking groups; homopolymers and copolymers. The carboxylic groups in the Carbopol molecule have affinity to water molecules and swells on hydration while remain insoluble in water (Singla, Chawla, Singh, 2000). This property makes the polymer valuable in the design of floating systems. Although HPMC and carbopol are used to develop floating drug delivery system, this study will explore the impact of individual as well as combination of polymer on the formulation properties such as swelling, drug release and *in-vivo* buoyancy.

Levofloxacin is an anti-biotic and second generation of fluoroquinolone. It is levorotatory isomers of ofloxacin. Levofloxacin has broad spectrum activity and used against gram positive and gram negative bacteria. It is used as second line therapy for the treatment of peptic ulcer disease in combination with omeprazole and amoxicillin where the first line triple therapy (lansoprazol, amoxicillin and clarithromycin) is failed (Blondeau et al., 2000; Verma et al., 2016). The effectiveness of this regime may be improved if levofloxacin is administered as gastro retentive drug delivery system. During the recent years different floating systems of levofloxacin has been investigated such as floating tablets, beads, swellable capsules and mucoadhesives gastroretentive systems (El-Zahaby, Kassem, El-Kamel, 2014a,b; Patil, Tiwari, Repka, 2016).

In the present study the floating tablets of levofloxacin using a systematic formulation design approach were developed and evaluated for pre-compression parameters of powder mixture, and post compression parameters of the tablets. The floating

behavior was investigated using individual polymers and combination of polymers. The compatibility study of levofloxacin with other polymers was investigated by FTIR (Fourier transform infrared spectroscopy), DSC, TGA and XRD. The drug release profile of tablet formulations containing individual and combination of polymers was investigated using various kinetics models like zero, first and Higuchi model to develop an understanding of matrix structure. The *in-vivo* floating studies were performed by administering optimized formulation containing X-ray grade barium sulphate to rabbits to confirm prolong gastric residence.

MATERIAL AND METHODS

Material

Levofloxacin hemihydrate was received as a gift sample by Nabi Qasim Industries (Pvt) Ltd, Karachi, Pakistan. HPMC-K4M, carbopol-940 polymer, poly vinyl pyrrolidone (PVP) was purchased from Sigma-Aldrich, Germany. Magnesium stearate was purchased from Merck Darmstadt, Germany. Sodium bicarbonate and citric acid was purchased from Sigma-Aldrich, USA. Lactose was purchased from Duksan Pure Chemicals, Korea. All other chemicals and solvent used in this study were of analytical grade.

Optimization of Gastro Retentive Levofloxacin Floating Tablets

Trial batches (F1 to F6) were prepared to screen the concentration of HPMC-K4M, Carbopol-940 polymer (Table I). Further optimization was performed using 2³ factorial design. The design was applied to establish the interrelationship between the selected variables (HPMC-K4M and Carbopol-940) polymer (Table II). The design includes 2 factors; each evaluated at 3 levels, i.e. lowest, middle, and highest concentrations of each variable, respectively (Table II). Nine batches were formulated (F7-F15) as per the factorial design and were analyzed for buoyancy, swelling index and drug release. The dependent variables swelling index (X1) and percentage drug release (X2) were used to optimize

the concentration of independent variables using design expert package 6.

TABLE I - Composition of the Gastro Retentive Levofloxacin Floating Tablets during preliminary screening of polymers (HPMC K4 and Carbopol 940) as excipients

Formulation No.	Variable Excipients	
	HPMCK-4M (mg)	Carbopol-940 (mg)
F1	100	0
F2	150	0
F3,	200	0
F4	0	100
F5	0	150
F6	0	200

Constant API and Excipients: Levofloxacin (mg)= 250; PVP (mg) = 10; NaHCO₃ (mg) = 20; Citric acid (mg) = 10; Lactose (mg)= q.s

TABLE II - Design for Gastro Retentive Levofloxacin Floating Tablets during optimization of HPMCK-4M and Carbopol-940 (mg) composition

Run	Block	Factor 1	Factor 2
		A: HPMCK-4M (mg)	B: Carbopol-940 (mg)
F7	1	50	50
F8	1	75	100
F9	1	75	50
F 10	1	100	100
F11	1	100	50
F12	1	50	75
F13	1	75	75
F14	1	100	75
F15	1	50	100

Preparation of Gastro Retentive Levofloxacin Floating Tablets:

The gastro retentive levofloxacin floating tablets were prepared using compression method. Accurately weighed levofloxacin (API), HPMC K4M and/or Carbopol (polymers) and lactose (filler) were mixed thoroughly.

Granulation was performed by adding binder i.e. poly vinyl pyrrolidone and passing the mass through a screen of sieve no. 20. The granules were dried at 70 in the thermo static hot air oven, ground in mortar and passed through a sieve No. 45. Then magnesium stearate (lubricant), sodium bi-carbonate, and citric acid (effervescent materials) were added to the above granules. Compression was carried out at a load of 800 kg using single punch machine (Pharmatest, Germany) All formulation batches of 100 tablets were prepared in this study.

Evaluation of Pre -compression Parameters

Bulk properties of the powdered mass (~100g) were calculated by measuring bulk density, tapped density angle of repose, Carr's index and Hausner's ratio. Bulk density was measured by placing the granules into the measuring cylinder and bulk volume and weight of powder mixture was measured and then it was calculated by using the following formula (Gharti et al., 2012)

$$pb = \frac{M}{V_b} \quad \text{Equation 1}$$

Where, b=bulk density, M=mass of granules, V_b=bulk volume

Tapped density is the ratio of total mass of powder to the tapped volume (V_t) of the powder. Tapped volume is measured by tapping the powder to a constant volume and it is calculated by following formula.

$$pb = \frac{M}{vt} \quad \text{Equation 2}$$

Where, =tapped density, M=Mass of granules, V_t= tapped volume of powder

Angle of repose was measured by passing the powder mixture into the funnel and height and radius of the pile was determined. And angle was determined by using the following formula (Wong et al., 2000)

$$\tan\theta = h/r \quad \text{Equation 3}$$

$$\theta = \tan^{-1} \left(\frac{h}{r} \right) \quad \text{Equation 4}$$

Where, h=height of pile r=radius of pile base

Carr's Index (I) measures the powder flow by using the formula

$$I = \frac{\rho t - \rho b}{\rho t} \times 100 \quad \text{Equation 5}$$

Where, ρt = Tapped density, ρb = Bulk density

Hausner's Ratio is the ratio of tapped density and bulk density.

$$\text{Hausner ratio} = \frac{\rho t}{\rho b} \quad \text{Equation 6}$$

Evaluation of Post-compression Parameters

Compressed tablets were characterized for weight variation, crushing strength, thickness, diameter and friability (Venkateswarlu, 2016).

Weight Variation: The weight variation of the tablets was performed by weighing 20 tablets selected at random and percent deviation from average weight was determined. According to USP if the average weight of tablet is more than 250 mg the % deviation should be in the range of ± 5 to $\pm 10\%$ (Patel, Patel, Yeole, 2005)

Content Uniformity: The content uniformity test was performed to ensure an acceptable concentration of drug is present in the tablets. Twenty tablets were weighed, crushed and powdered mass equivalent to 250 mg of levofloxacin was dissolved in 100 ml of 0.1N HCl, and filtered. A standard solution of drug was prepared using 250 mg levofloxacin. From these solutions, 5 ml was diluted to 50 ml by adding 0.1N HCl and the drug content was estimated by using UV visible spectrophotometer at 287nm (Patel, Patel, 2006)z

$$\text{Drug content (\%)} = \frac{G(\text{actual})}{G(\text{powder})} \times 100 \quad \text{Equation 7}$$

Hardness Test: Tablet hardness was determined by recording the crushing strength of individual tablets N=20 selected at random from each batch. The results were described as average \pm standard deviation.

Friability Test: Weight loss from the tablets following 100 revolutions on Roche friabilator was calculated by following formula.

$$\text{Friability} = (W_o - W_t) / W_o \times 100 \quad \text{Equation 8}$$

Where, W_o = initial weight, W_t =final weight. According to USP the friability should be less than 1%.

In Vitro Buoyancy Test:

The in vitro buoyancy was determined from floating lag time and floating time. This test was performed by introducing the tablet to 900 ml of 0.1N HCl (pH 1.2) maintained at 37. The results were described in term of floating lag time (FLT) and total floating time (TFT) (Patel, Patel, Yeole, 2005; Patel, Patel, 2006; Patil, 2015). Time required for the tablet to raise on the surface was taken as floating lag time (FLT). Time up to which the tablet remained buoyant is determined as total floating time (TFT).

Dissolution Release Profile:

The drug release was performed using USP type II dissolution apparatus containing simulated gastric fluid having pH 1.2 adjusted using 0.1 N HCl maintained at 37 ± 0.5 operated at 100 rpm (Raghavendra Rao et al., 2010). At discrete time intervals, 5 ml of the sample was withdrawn and the medium was replaced with equivalent volume of blank (simulated gastric fluid having pH 1.2 adjusted using 0.1 N HCl). The drug solution was further diluted and analyzed at 287nm by using UV spectrophotometer.

Swelling Index:

Levofloxacin floating tablets were weighed individually, placed separately in glass beaker containing 25 ml of 0.1 N HCl (pH 1.2) and incubated at 37 °C±1°C. At regular time interval floating tablets were removed from beaker and excess surface liquid was removed carefully using the blotting paper. The swollen floating tablets were then re-weighed and swelling index (SI) was calculated using the following equation (Patel et al., 2009; Raghavendra Rao et al., 2010).

$$\text{Swelling index} = \left(\frac{\rho t - \rho b}{\rho t} \right) \times 100 \quad \text{Equation 9}$$

Where, W_t = weight of tablet after swelling, W_0 = weight of tablet before swelling

Release Kinetic of Drug:*Zero Order Release Rate Kinetic Model:*

To analyze the *in-vitro* release behavior various kinetics models were used to describe release kinetics. The zero order release kinetics describes the system where drug release is independent of its concentration. The equation used for analysis is as under (Hadjioannou et al., 1993)

$$F = K_0 t \quad \text{Equation 10}$$

Here, F = fraction of drug release, K_0 = rate constant, t = time release

First Order Model:

The first order describes the release from system where release rate is concentration dependent. The system is expressed by the following equation

$$\log C = \log C_0 - Kt/2.303 \quad \text{Equation 11}$$

Here, C_0 is the initial concentration of the drug, K is the first order rate constant, t is the time

Higuchi Release Model:

The Higuchi model describes the release of drug from insoluble matrix as a square root of time dependent process based on following equation (Higuchi, 1963)

$$F = K_H \cdot t^{1/2} \quad \text{Equation 12}$$

Here, F is the amount of drug release, K_H is the constant, t is the release time

Korsmeyer and Peppas Model:

Korsmeyer and Peppas model described a relationship of drug release from a polymeric system using following equation;

$$Mt/M_\infty = K_M t^n \quad \text{Equation 13}$$

Mt/M_∞ is the fraction of drug release, K_M is the release rate constant, t is the release time, n is the release exponent. "n" value is used to characterize different release mechanism of drug as describe as if $n < 0.45$ Fickian diffusion, $0.45 < n < 1$ Non Fickian diffusion, $n = 1$ Case II transport and $n > 1$ Super case II transport

Attenuated Total Reflectance Fourier transform infrared (ATR-FTIR)

ATR FT-IR spectra were obtained for the pure drug (levofloxacin), polymer (HPMCK-4M and Carbopol-940) and optimized formulation in the range of 4000–500 cm^{-1} . Each sample was placed in the light path of sample cell and the spectrum was recorded (Nicolet Instrument Co.).

Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) (Pyris™ DSC 6000; PerkinElmer, Waltham, MA) was performed to analyze the thermo-physical properties of pure drug (levofloxacin), polymer (HPMCK-4M and Carbopol-940) and optimized formulation. The accurately weighed samples were crimped in standard aluminium pans and heated from 20°C to 250°C at a rate of 10°C/minute

under constant purging of N₂ at 10 mL/minute. An empty pan, sealed in the same way as the sample, was used as a reference.

Thermogravimetric Analysis (TGA)

Gravimetric changes in the pure drug (levofloxacin) HPMC K4 M, Carbopol 940 and optimized formulation were recorded by heating at 10 °C/min over a temperature range 25-400 °C using TGA equipment (Perkin Elmer USA). The instrument was previously calibrated for weight and heat flow measurements.

In-vivo X-Ray Imaging Studies

An *in vivo* animal study was performed in albino rabbits (having 3 rabbits in each group, n=3) using X-ray imaging technique (Medford). for evaluating the floating potential of tablet formulation. The study protocols were approved from departmental Animal Ethical Committee BZU (47/PEC dated 2-1-2018). The animals (rabbits) (weighing of 2-2.2 Kg were housed under standard laboratory conditions at 25±2 °C and 55±5% RH with standard diet and tap water *ad libitum*. Prior to the start of the studies, the rabbits were kept overnight under fasting condition in order to avoid difficulties during imaging. X-ray image of the empty stomach was taken in rabbits before experimentation. The rabbits (n=3) were orally administered with the optimized levofloxacin

floating tablet containing 50 mg barium sulphate. The rabbits were placed in the upright position for imaging to locate the position of both control and floating tablets in the GI tract under X-ray machine (M/s Siemens 300 MA with fluoroscopy, München, Germany) at different time intervals like 0, 2, and 4 hours, respectively (Bansal et al., 2016).

Statistical Analysis;

The resulting data were analyzed by using Microsoft Excel 2010 by applying one-way ANOVA. Differences between formulations were considered to be significant at $p \leq 0.05$.

RESULTS AND DISCUSSIONS:

Pre-Compression Parameters of Levofloxacin Granules:

Bulk density of the granular mass was recorded in the range of 0.45-0.54 g/ml which increased to 0.6 g/ml after tapping. Approximately 10-15% increase in density was explained with improved granular packing following tapping. The results of parameters angle of repose < 40°, Hausner ratio <1.5 and Carr's index <15 indicate good flow of the granular mass (Table III). From these results one can reliably predict uniform die filling during the compression and uniform weight of compressed tablets.

TABLE III - The pre-compression flow properties of granules of levofloxacin

Formulation No.	Bulk Density (g/ml)	Tapped density (g/ml)	Carr's Index (%)	Hausner Ratio	Angle of Repose (°)
F1	0.54	0.60	10	1.111	33.0
F2	0.54	0.60	10	1.111	33.4
F3	0.53	0.60	11.2	1.132	33.1
F4	0.54	0.60	10	1.111	33.0
F5	0.54	0.60	10	1.111	33.4

(continues on the next page...)

Formulation No.	Bulk Density (g/ml)	Tapped density (g/ml)	Carr's Index (%)	Hausner Ratio	Angle of Repose (°)
F6	0.35	0.40	12.5	1.142	36.0
F7	0.33	0.39	15.3	1.181	36.4
F8	0.35	0.41	14.6	1.171	36.3
F9	0.34	0.40	15	1.170	36.4
F10	0.35	0.41	14.6	0.851	37.0
F11	0.48	0.53	9.68	1.107	35.5
F12	0.48	0.55	11.8	1.134	34.5
F13	0.45	0.51	8.70	1.095	34.1
F14	0.45	0.50	9.92	1.112	31.0
F15	0.43	0.47	8.31	1.091	33.7

Post-compression Parameters of Gastro Retentive Levofloxacin Floating Tablets

The compressed tablet containing HPMC and polymeric combinations (i.e. HPMC and carbopol) had hardness 4-6 kg/cm³ while the formulations containing carbopol alone were harder than the counterparts containing HPMC. The weight variation results of

compressed tablets were found within the compendia limits i.e < 5% (Table IV). The content uniformity results reflect drug concentration within 1.5% of label claim. These results provide reasonable evidence that the formulation complies with compendial requirements. Therefore, the formulations were further investigated for performance parameters such as *in vitro* swelling and drug release.

TABLE IV - The post compression parameters of Gastro Retentive Levofloxacin Floating Tablets.

Formulation No.	Thickness (mm)	Hardness Kg/cm ³	Diameter (mm)	Weight variation (mg)	Friability (%)	Drug Content (%)
F1	2.95+0.3	4.1+0.05	12.9	486+1.3	>1	98.50+3.23
F2	2.95+0.3	4.0+0.06	12.8	487+1.56	>1	99.21+2.52
F3	3.0+0.3	4.6+0.10	13.0	487+1.56	0.9	99.35+2.54
F4	2.9+0.3	4.5+0.12	12.9	487+1.50	0.8	98.6+2.76

(continues on the next page...)

Formulation No.	Thickness (mm)	Hardness Kg/cm ³	Diameter (mm)	Weight variation (mg)	Friability (%)	Drug Content (%)
F5	2.9+0.3	4.0+0.09	12.8	486+1.39	0.8	98.56+2.97
F6	3.0+0.01	8.5+0.14	12.8	487+1.52	0.15	99.25+1.56
F7	3.0+0.01	8.2+0.09	12.8	486+0.82	0.50	99.47+2.39
F8	2.85+0.2	8.5+0.14	12.9	487+1.26	0.35	99.54+2.64
F9	2.9+0.35	8.5+0.01	13.0	487+1.76	0.37	98.75+2.85
F10	3.0+0.01	8.4+0.04	12.9	487+1.56	0.52	99.67+2.56
F11	3.0+0.3	5.1+0.09	12.8	486+1.35	0.32	98.74+1.97
F12	2.9+0.3	5.4+0.15	13.0	487+1.13	0.35	98.43+2.92
F13	3.0+0.01	5.1+0.03	12.9	487+1.72	0.34	97.47+2.45
F14	2.95+0.3	6.0+0.10	12.8	486+1.2	0.50	99.63+3.05
F15	2.9+0.3	5.5+0.10	12.8	487+1.54	0.17	97.42+2.97

In-vitro Buoyancy Studies:

Floating lag time (FLT) was determined to describe the time delay with which a dosage form floats onto the gastric fluid. Both the degree of polymer wetting and effervescence contribute to the floating of dosage form. Floating lag time (FLT) of formulations containing HPMC-K4M (F1 to F3) was 15 to 42 sec whereas the carbopol-940 (F4 to F6) had FLT 300 to 600 sec. Formulations containing both type of polymers showed FLT in the range of 60 to 350 sec. Among them the F11 showed minimum FLT i.e., 60 sec and was considered optimum formulation (Dudhipala et al., 2016) in vitro and ex vivo evaluation

title><secondary-title>J Bioequiv Availab</secondary-title></titles><periodical><full-title>J Bioequiv Availab</full-title></periodical><pages>118-24</pages><volume>8</volume><number>3</number><dates><year>2016</year></dates><urls></urls></record></Cite></EndNote>. The total floating time (TFT) was observed between 4 to 24 hrs. The results indicate that the formulations prepared using HPMC-K4M (F1 to F3) showed shorter TFT (< 4 h) as compared to the carbopol-940 (F4 to F6) therefore, exhibits shorter TFT (8h). The formulations containing both polymers (F7 to F15) had TFT (>16 h) (Figure 1 and 2) (Bahri-Najafi et al., 2017; Porwal, Dwivedi, Pathak, 2017).

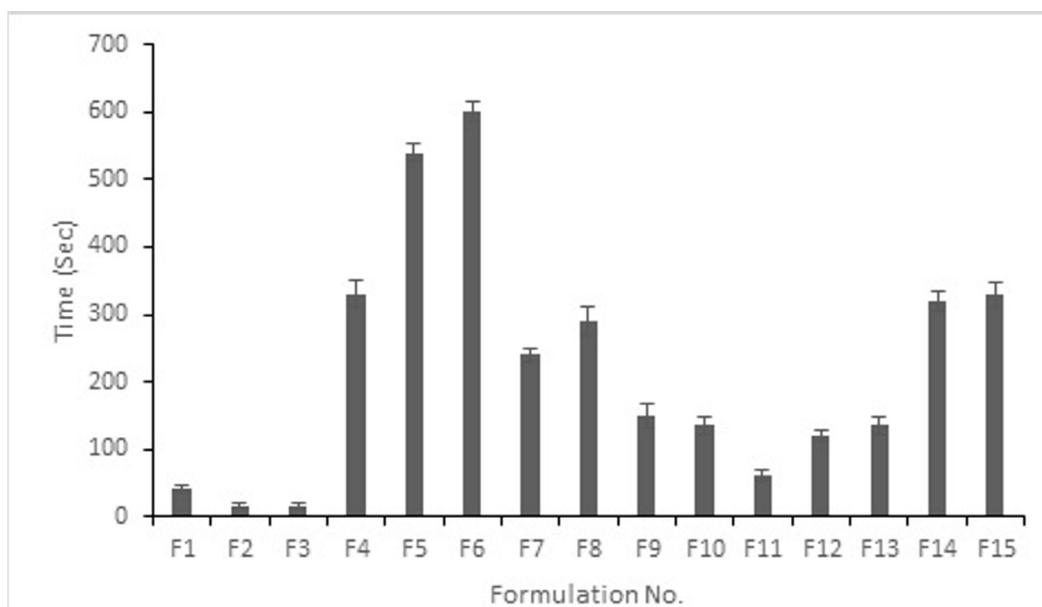


FIGURE 1 - In vitro buoyancy profile (floating lag time) of levofloxacin floating tablets (F1-F15).

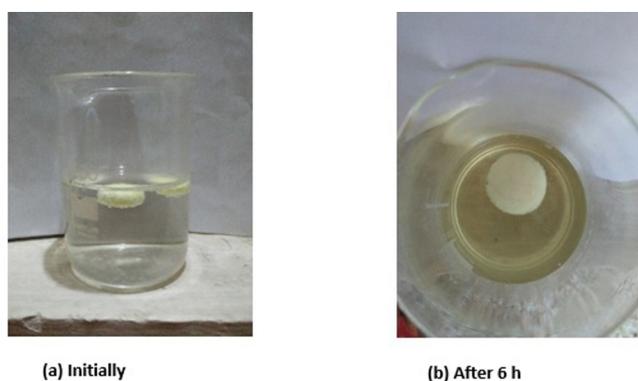


FIGURE 2 - Water uptake study of levofloxacin tablets containing HPMC-K4M

Swelling Index

The swelling behavior is the fundamental characteristics to ensure buoyancy and drug release from matrix tablets. The floating tablets composed of HPMC-K4M/ or carbopol-940 build a gel layer when they come in contact with buffer *in-vitro* and biological fluid *in-vivo*. This gel layer facilitated the release of drug. The figure 3 A, B shows the swelling behavior of HPMC-K4M (F1 to F3) and carbopol-940 (F4 to F6), respectively (Vemula, Venisetty, Veerareddy, 2017). The

swelling of floating tables containing HPMC-K4M (F1 to F3) and carbopol-940 (F4 to F6) was increased with the concentration HPMC-K4M and carbopol-940 (i.e. from 100 to 200 mg while keeping other components constant), respectively, upto seven hour of study period. This effect was due to increase in hydration capability of hydrophilic polymers when their concentration was also increased.

The swelling behavior of selected formulation containing combination of both HPMC-K4M and carbopol-940 (F7 to F15) is presented in figure 3C. The formulations i.e. F7 and F10 contain HPMC: Carbopol at 1:1 however, the total polymer concentration was 100 and 200 mg, respectively. The formulation F11 and F15 contain HPMC K4M and Carbopol 940 at ratios 2:1 and 1:2 respectively, with total polymer concentration of 150 mg (Essa et al., 2015).

The results revealed that formulation (F7) had shown lesser swelling as compared to the F10. This effect was possibly due to concentration dependent water absorption by the polymer. The formulation F15 showed higher swelling as compared to F11. Difference in the swelling amongst these formulation was linked with the concentration of carbopol in the formulation.

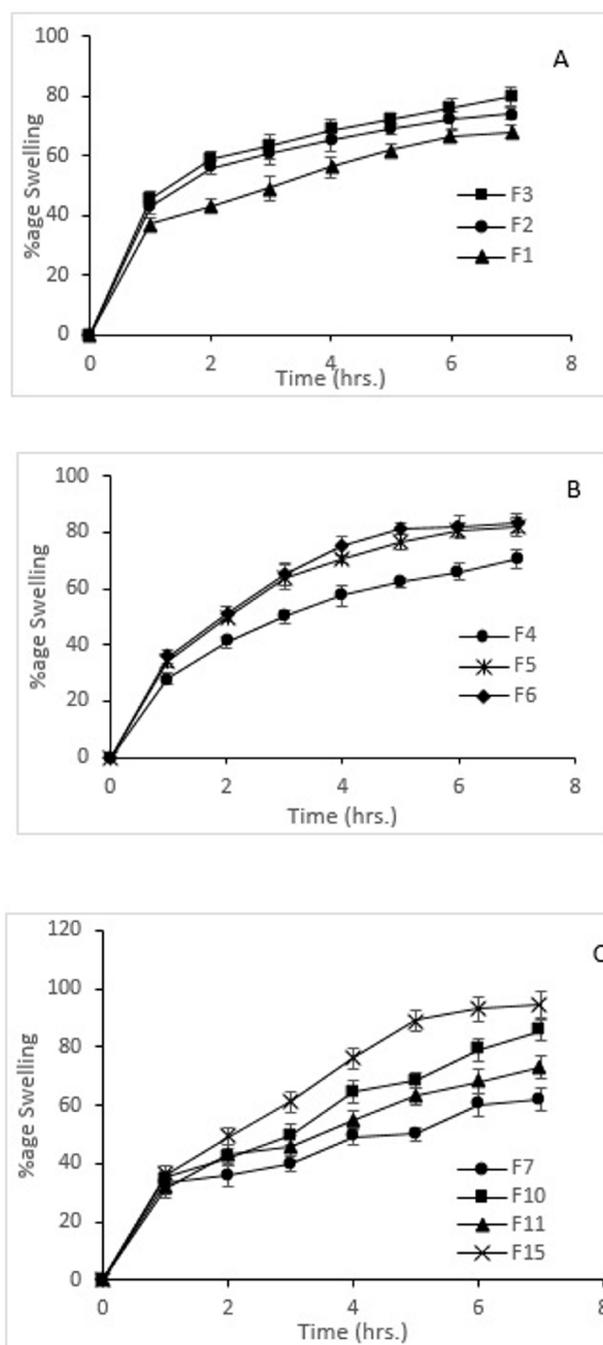


FIGURE 3 - Swelling Index profile of formulations(A) (F1-F3) containing HPMC-K4M, (B) (F4-F6) containing carbopol-940 (C) (F7-F12) containing HPMC-K4M and carbopol-940

Dissolution Study of Gastro Retentive Levofloxacin Floating Tablets

The dissolution profile of gastro retentive levofloxacin floating tablets prepared using varying

contents of HPMC-K4M (F1 to F3) is presented in Figure 4 A. The drug release was recorded 78.5%, 48.1% and 28.5 % for the formulations F1, F2 and F3 respectively. There was significant decrease in % levofloxacin release in first hour among three investigated HPMC-K4M concentrations in the formulation. Subsequently, at the end of 6 hour of release study period, these formulations showed the release of 90.0%, 82.3% and 82.6%.

The release profile of gastro retentive levofloxacin floating tablets (F4 to F6) prepared using varying Carbopol contents are described in figure 4B. The drug release was recorded as 49.6 %, 44.5 % and 27.1 %, for F4, F5, F6, respectively, after 1h. Subsequently, at the end of 6 h of the study period, these formulations showed the release of 75.3, 93.9 and 93.5 %. These results indicated that increasing the HPMC-K4M or Carbopol concentration in gastro retentive levofloxacin floating tablets, had significantly decreased % levofloxacin release in first hour. However, there was no significant difference in the total amount of levofloxacin release after 6 hour of release study period.

Based on these results, 100 mg of individual polymer (HPMC-K4 or carbopol 940) was considered optimum. In the next stage, formulations prepared using combination of the retardant polymers were compared with the counterparts containing individual polymer in terms of drug release rates profile.

The formulations F7 to F15 were prepared using software directed combination of HPMC-K4M and Carbopol. The dissolution profiles of selected formulation are presented in Figure 4 (C and D). The comparison of % levofloxacin release profile from formulation F7 and F10 is presented in Figure 4 (C). These formulations were having HPMC-K4M and Carbopol at 1:1 ratios while the total amount the polymer was 100 and 200 mg for F7 and F10, respectively. The results showed that during first hour of release the F7 and F10 showed 43.8% and 31.0% release, respectively. After 6 h the drug release was observed 94.2 and 88.1 for these formulations.

The comparison of % levofloxacin release profile from formulation F11 and F15 is presented in Figure 4 (D). The formulation F11 and F12 contain HPMC-K4M and carbopol at 2:1 and 1:2 ratios, respectively with

total polymer contents 150mg. The results showed that there was no significant difference in % levofloxacin release during first hour. However, the formulation F11 showed better % age release profile compared to F15. These results indicated that higher ratio of HPMC-K4M in the formulation (F11) had shown improved % levofloxacin release.

These results indicated that when both (HPMC-K4M and Carbopol) polymer are used in combination, the lower concentration of total polymer showed higher percentage of levofloxacin release.

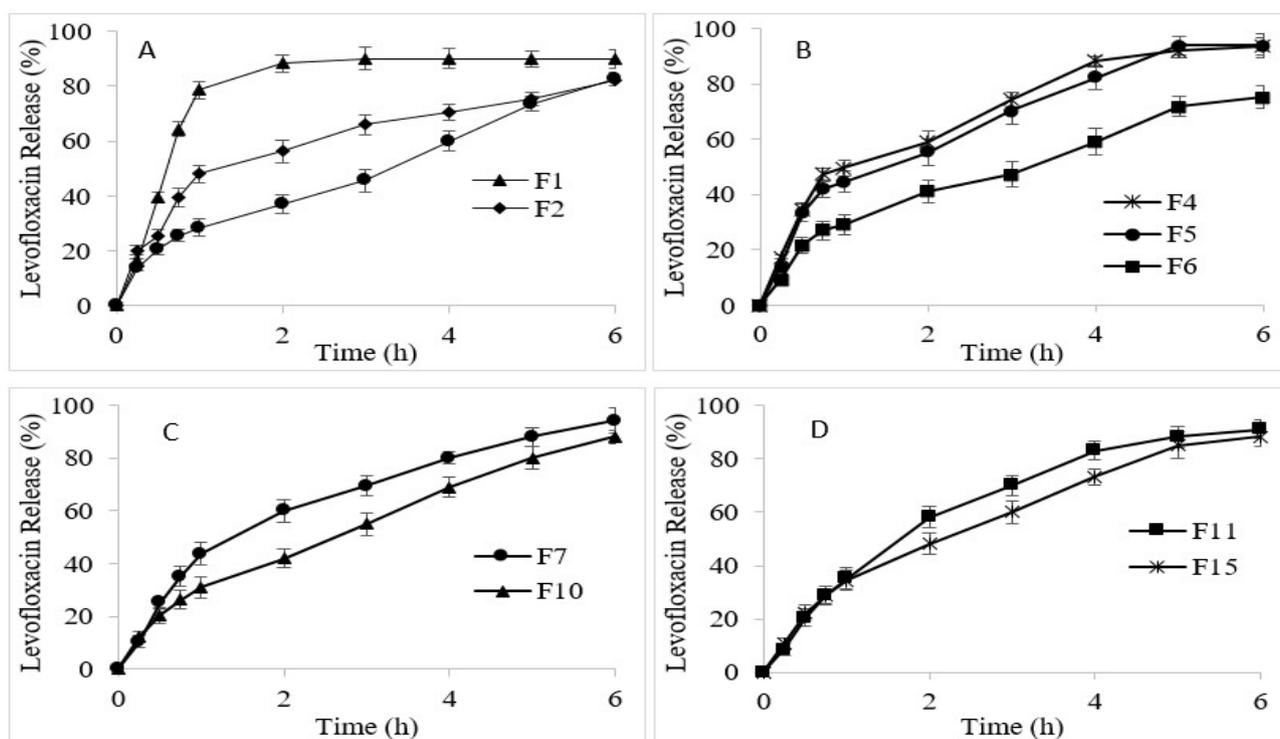


FIGURE 4 - Dissolution profile of (A) formulation (F1-F3) containing HPMC-K4M, (B) formulation (F4-F6) containing Carbopol (C and D) formulation containing HPMC-K4M and carbopol-940

Drug Release Kinetics

It is described that the drug release from formulation F1 followed first order release kinetics ($R^2=0.974$) while the F2 counterparts was explained by Higuchi equation ($R^2=0.983$). The drug release from F3 was described reliably by zero order release kinetics ($R^2=0.982$) (Table VI). Possible reason to this effect may be difference in the porosity of the gel matrix formed after the swelling of polymer(s) (Bettini et al., 1994; Panda, Subhadarsini, Mallick, 2016).

TABLE V - Interpretation of FTIR spectra of polymers and formulations

Material	Peak (cm-1)	Functional
levofloxacin	1719.35	Strong C=O Stretching
	1618.85	Strong C=C Stretching
	1538.48	Strong C-N Stretching

(continues on the next page...)

Material	Peak (cm-1)	Functional
HPMC-K4M	1619.43	Strong C=C Stretching
	1469.71	Medium C-H bending
	1454.20	Medium C-H bending
Carbopol-940	1698.27	Strong C=O Stretching
	1620.18	Strong C=C Stretching
	1453.91	Medium C-H bending
Formulation 3	1720.32	Strong C=O Stretching
	1619.31	Strong C=C Stretching
	1539.36	Strong C-N Stretching
Formulation 6	1719.49	Strong C=O Stretching
	1619.13	Strong C=C Stretching
	1539.29	Strong C-N Stretching
Formulation 11	1719.49	Strong C=O Stretching
	1619.29	Strong C=C Stretching
	1539.29	Strong C-N Stretching

The drug release from formulation F4 followed first order kinetics with R² value of 0.969 while the formulations F5 and F6 were explained by zero order kinetics (with R² value of 0.963 and 0.986 respectively) after a time lapse of 1 hour. In vitro release profile of formulations prepared using combination of HPMC K4 and carbopol 940 (F7 to15) followed Higuchi model with R² value >0.98. The drug release mechanism from the formulations containing both polymers i.e. HPMC K4 and carbopol 940 was further explored using Korsmeyer-Peppas model and the results are presented in Table VII. All of the formulations (F7-15) showed n value > 0.5 indicating non-fickian diffusion mechanism of drug release. These results suggest the involvement of both the

swelling and diffusion controlled drug release mechanism form these formulations.

TABLE VI - Drug release kinetic models of formulations F1-F15

Formulation No.	Zero Order		First Order		Higuchi Model	
	K0	R	K1	R	KH	R
F1	15.221	0.9736	0.242	0.9584	31.194	0.9566
F2	17.086	0.9654	0.321	0.9673	35.232	0.9833
F3	16.435	0.9815	0.339	0.9684	34.554	0.9405
F4	19.904	0.8702	0.780	0.9690	43.286	0.9650
F5	21.616	0.9633	1.745	0.6284	46.759	0.7876
F6	20.253	0.9863	0.412	0.9760	38.309	0.9580
F7	15.285	0.9782	0.262	0.9727	31.199	0.9821
F8	21.754	0.9494	0.506	0.9807	41.546	0.9919
F9	19.381	0.8302	0.607	0.8841	41.473	0.8959
F10	24.388	0.9012	0.542	0.9603	42.280	0.9753
F11	18.663	0.9319	0.468	0.9793	39.238	0.9833
F12	18.358	0.9624	0.405	0.9928	37.880	0.9923
F13	18.417	0.9790	0.386	0.9916	37.678	0.9921
F14	18.995	0.9875	0.337	0.9833	35.170	0.9847
F15	16.247	0.9741	0.309	0.9934	33.407	0.9944

Product Optimization Studies

Analysis of responses (swelling, drug release) from the experimental matrix (Raghavendra Rao et al., 2010) confirms that the results on drug release can be explained by a 2 factor equation

$$\text{Sqrt}(\% \text{ Released}) = 9.42 + 0.19 * A[1] - 0.023 * A[2] + 0.19 * B[1] + 0.012 * B[2]$$

Model equation is reliable as it measure correlation coefficient value 0.94 and signal to noise ratio value 12 i.e. greater than 4. The F-value of 16.22 implies that the model is significant. There is only a 0.97% chance that F-Value could occur due to noise.

The drug release is precisely described as a function of concentration of both carbopol and the HPMC. The result indicated that the release rate decrease exponentially with the increase in HPMC fraction and for the carbopol a linear increase in drug release was

recorded. The slope factor for each concentration of variable polymer is influence, although to a lesser extent, by the polymer at fixed concentration. Variation in slope factors suggest an interaction between the input variable in the output/response (Figure 5).

The swelling index was also analyzed as a function of polymer concentration. Model equation that fit he results is

$$\text{Sqrt}(\text{Swelling}) = +9.46 - 0.10 * A[1] + 0.1 * A[2] - 0.2 * B[1] - 0.18 * B[2]$$

Where A and B denotes HPMC and carbopol.

Statistical analysis of model fit results were less promising than the release profile. The value of parameters

correlation coefficient R^2 and signal to noise ratio was 0.79 and 4.9, respectively, suggesting the suitability of model. However, ANOVA results suggest that there is 11% chance that results could be due to noise. Another approach to validate the model is to predict a formulation with desired properties. In this stage an optimized formulation composition was derived which would have floating behavior and drug release 'maximum'. The in-silico tool predicted a formulation F11 with probability of occurrence 0.79. the swelling and release results for software predicted optimized formulation were in close agreement with the target values, therefore confirms the validity of the model in predicting the formulation behavior. The optimized formulation F11 was evaluated for further analysis and in-vivo buoyancy studies described in the sections below.

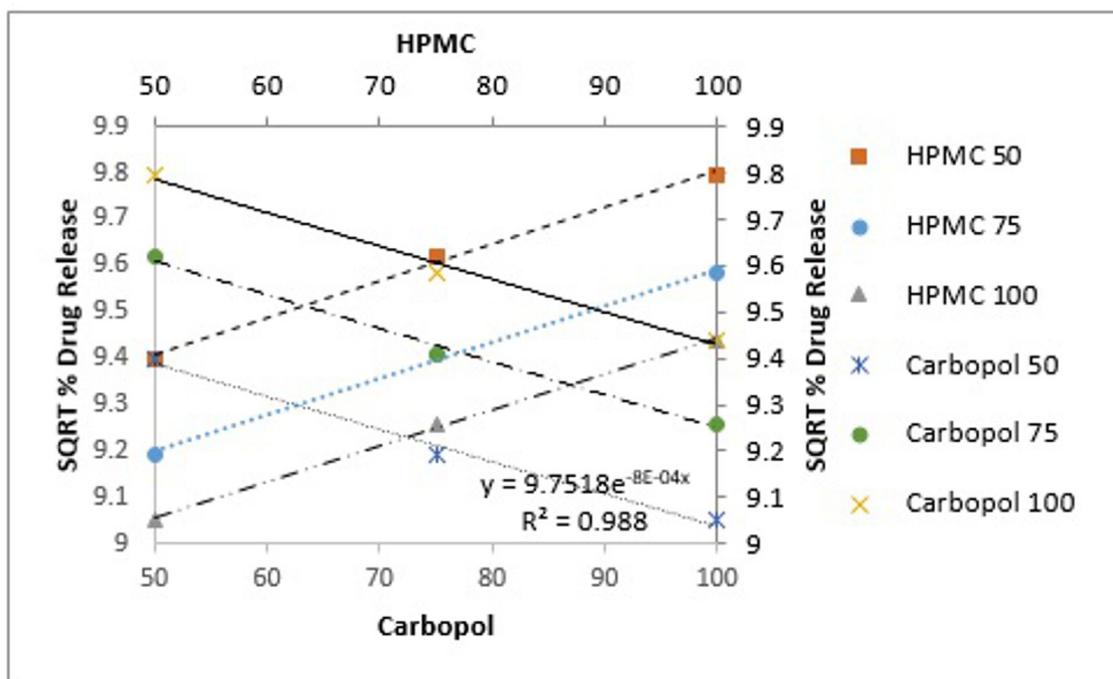


FIGURE 5 - Contribution of polymeric entities on drug release

FTIR Spectrum of Levofloxacin Polymers & Formulations

FTIR spectra of levofloxacin was shown in Figure 6 and the peaks are mentioned in the table V. According

to this peaks at 1719.35 cm^{-1} , 1618.85 cm^{-1} and 1538.48 cm^{-1} due to $-\text{COOH}$ group, $\text{C}=\text{C}$ group and $\text{C}-\text{N}$ groups respectively (El-Zahaby, Kassem, El-Kamel, 2014b).

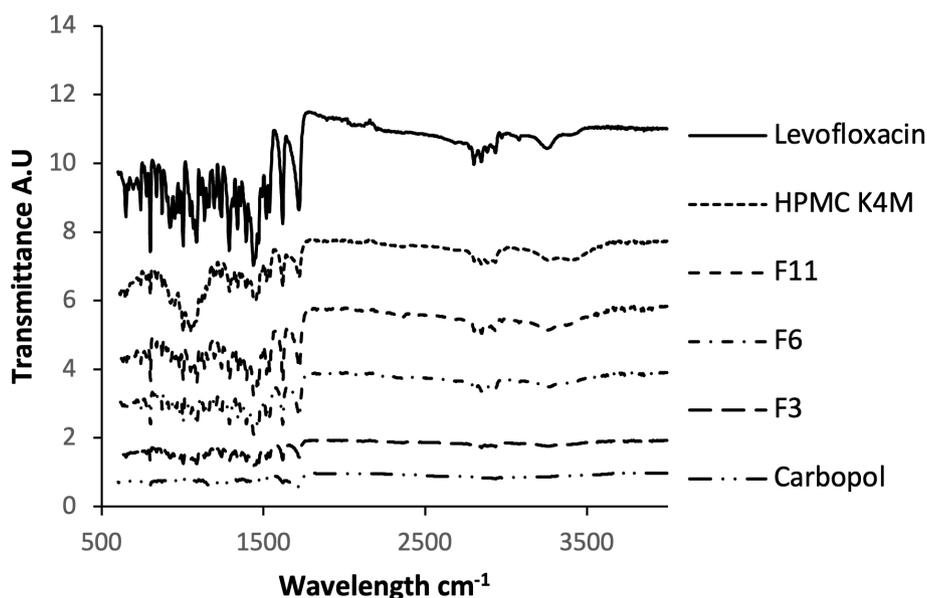


FIGURE 6 - ATR-FTIR spectra of levofloxacin, HPMC K4M, F11, F6, F3 and Carbopol

FTIR spectra of the polymers HPMC-K4M and carbopol-940 is shown in fig. Here different peaks are obtained due to respective functional group. According to table peaks at 1619.43 cm^{-1} , 1469.7 cm^{-1} and 1454.20 cm^{-1} belong to HPMC-K4M due to strong C=C stretching, medium C-H bending in alkane, and C-H bending in aldehyde respectively (8). Peaks at 1698.27 cm^{-1} , 1620.18 cm^{-1} and 1453.91 cm^{-1} belong to carbopol-940 polymer due to strong C=O stretching, strong C=C stretching in ketone and medium C-H bending in alkane respectively (8). When two polymers used in combination in levofloxacin tablets, results obtained are also shown in table 5. Peaks obtained in formulations are present at their own positions, at 1719.35 cm^{-1} , 1618.85 cm^{-1} , and 1538.48 cm^{-1} due to strong C=O stretching, strong C=C stretching and strong C-N stretching respectively. It indicates that there is no shifting of peaks, no interaction, no incompatibility was found in drug and polymers. So these polymers can be used in gastro retentive floating levofloxacin tablets.

Differential Scanning Calorimetry

DSC thermogram of HPMC, levofloxacin compressed tablet and carbopol are described in figure 7A. A broad endothermic peak with onset temperature $\sim 100\text{ }^{\circ}\text{C}$ in the thermogram of HPMC denotes dehydration

of water molecules (Zaini et al., 2017). DSC curve of the levofloxacin revealed a step like increase in the enthalpy at $56\text{ }^{\circ}\text{C}$ which relate to polymorphic transition (Gorman, Samas, Munson, 2012) while an endothermic peak at $105\text{ }^{\circ}\text{C}$ relates to loss of water from hemihydrate structure of the crystal lattice. Second endothermic peak observed at 219 accords to the melting of levofloxacin (Figure 7A).

Thermogram of Carbopol describe a broad endotherm at temperature $50\text{-}111\text{ }^{\circ}\text{C}$ followed by a step like increase in enthalpy at $130\text{ }^{\circ}\text{C}$ depicting glass transition in the polymer. Levofloxacin compressed tablets reproduce the thermal events recorded from the individual substances. Absence of any additional thermal event in the thermogram of tablet formulation confirms that the formulation components are chemically compatible as no chemical interaction was observed. Furthermore, the DSC result were found in line with the FTIR data.

TGA results of levofloxacin, compressed tablet formulation, carbopol-940 and HPMC-K4 showed a weight loss of 2, 5, 7 and 8% upto $100\text{ }^{\circ}\text{C}$ (Figure 7B). apparently these gravimetric changes were attributed to loss of moisture from these materials. Weight changes observed at higher temperature i.e. $>200\text{ }^{\circ}\text{C}$ were linked to phase transition as well as the thermal degradation of these samples. Nevertheless, all of these samples were thermally stable customary condition of processing and use.

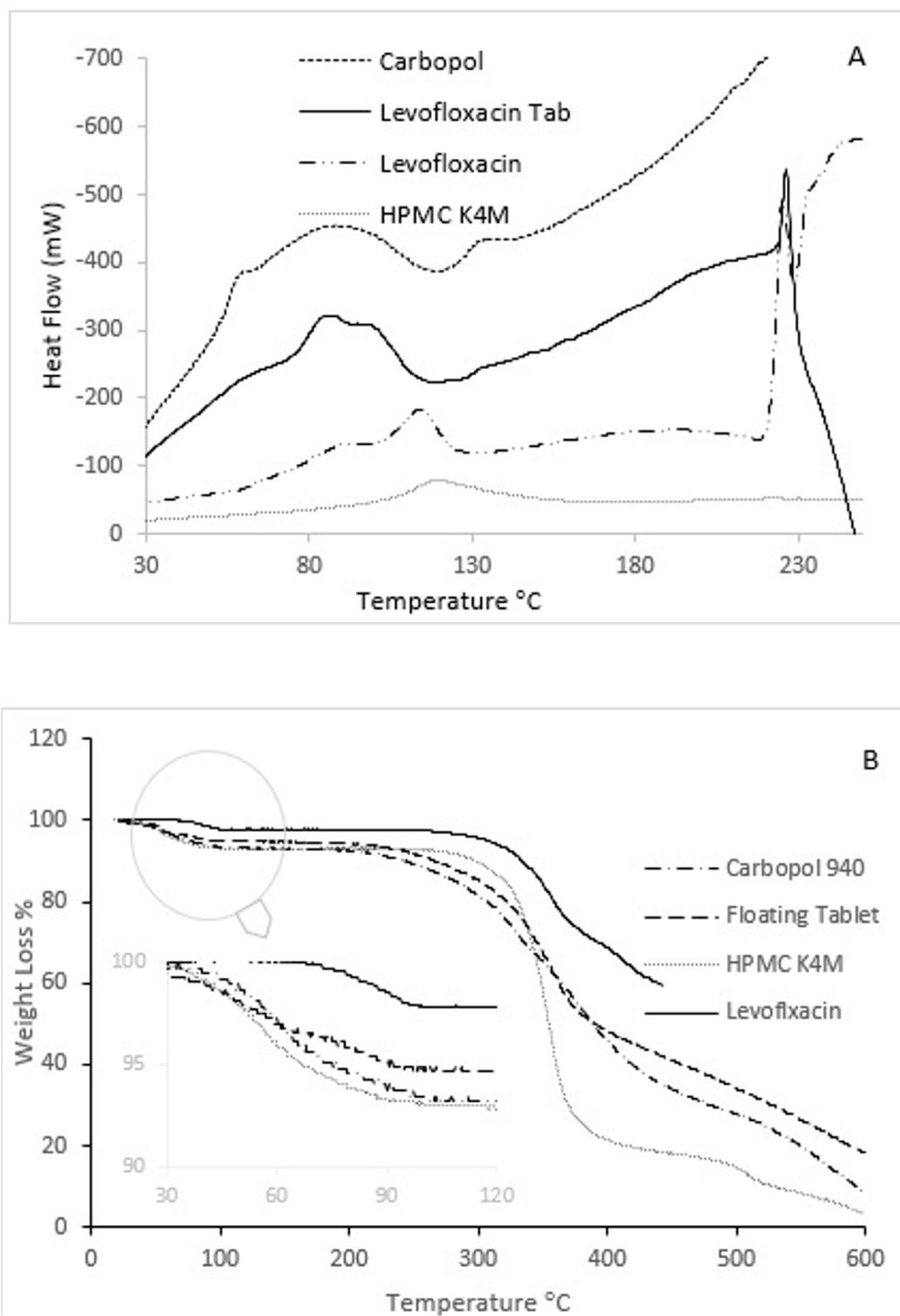


FIGURE 7 - (A) DSC thermograms for: (a) Carbopol-940, (b) Levofloxacin floating tablet (c) Levofloxacin (d) HPMCK4M.(B) TGA thermogram a) Carbopol,940 (b) Levofloxacin floating tablet (c) HPMCK4M (d) Levofloxacin

X-Ray Powder Diffraction (XRD)

XRD patterns of HPMC, levofloxacin compressed tablet and carbopol are described in figure 8AB. The XRD pattern of HPMC and carbopol did not describe sharp peaks over the measurement range, suggesting amorphous nature of these substances. The pattern

of levofloxacin, on the other hand showed a sharp diffraction peak at 25 degrees along with other small diffraction peaks signifies a crystalline nature of the material. The XRD pattern of levofloxacin formulation closely assimilates with the pure drug, suggesting native form of the drug is retained following its compression.

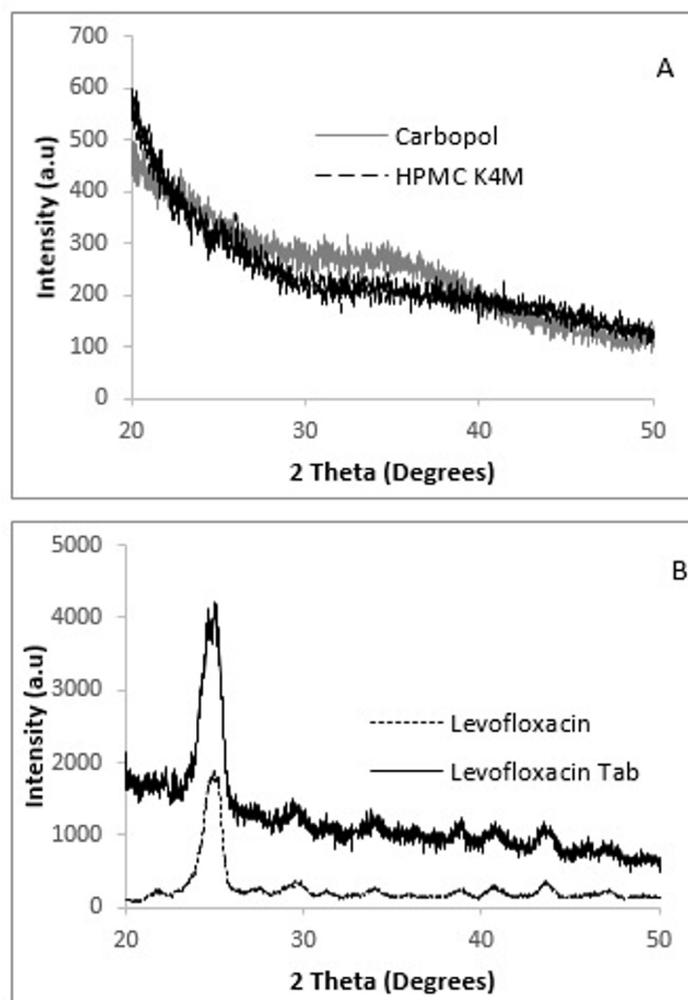


FIGURE 8 - XRD spectra of (A) Carbopol and HPMC K4M (B) Levofloxacin and Levofloxacin Tablet

In-vivo buoyancy of selected formulation studies using X-ray Imaging

The prepared floating tablets (F11) were selected for gastric retention using X-ray imaging. Figure 9 shows the gastric retention of floating tablet in rabbits after 0, 2 and 4 hours. The *in-vivo* buoyancy of floating tablet was

confirmed by X-ray imaging at regular time interval after ingestion of floating tablet containing barium sulphate (Diós et al., 2016). The behavior of the floating tablet in the rabbit's stomach was observed using radiographic imaging technique. The floating tablet seen in the stomach of rabbit till 4 hour (n=3) showed the confirmation of buoyancy of the floating tablet

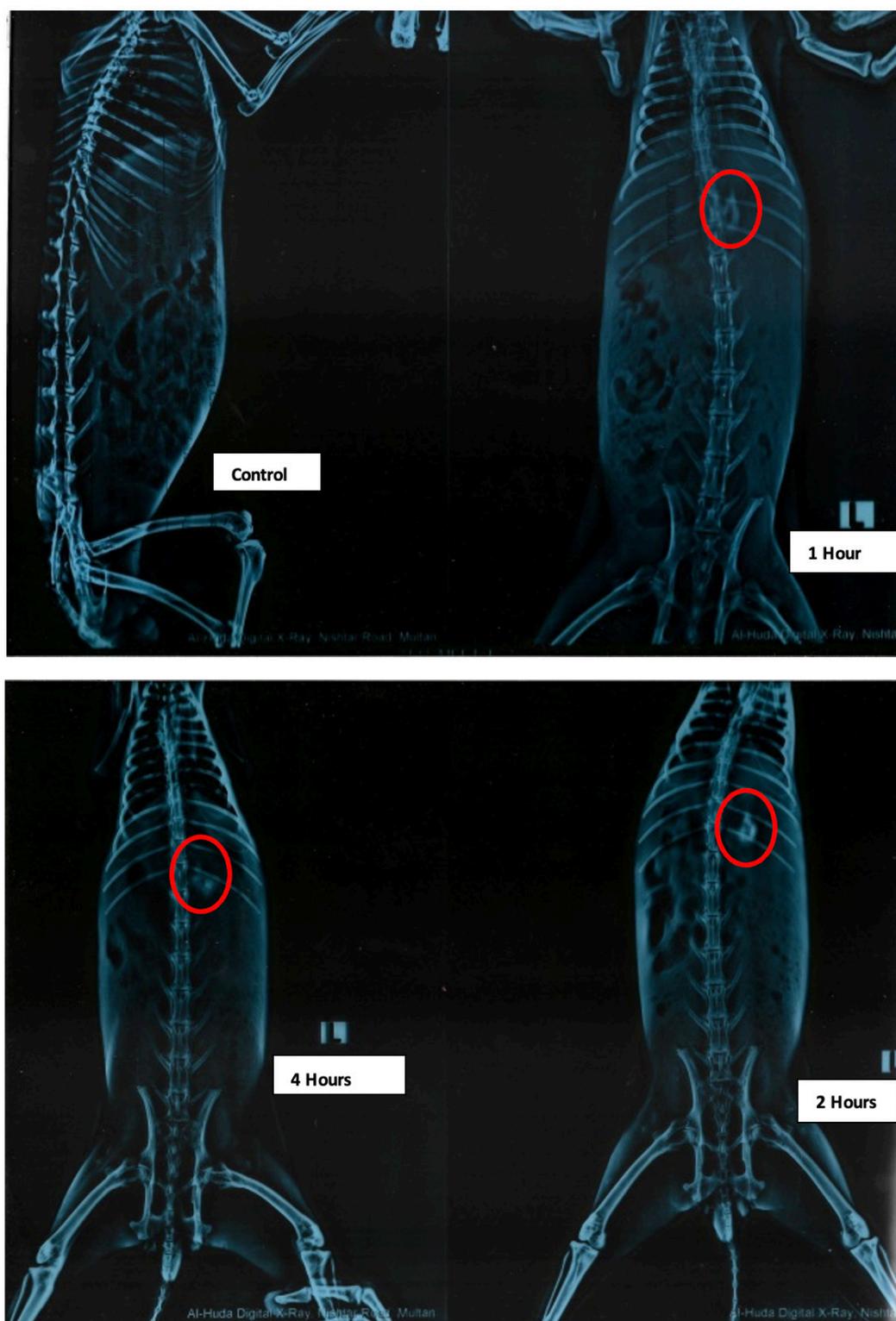


FIGURE 9 – *In vivo* buoyancy of selected formulation studies using X-ray Imaging

CONCLUSION:

The gastro retentive floating levofloxacin tablets were successfully developed using different concentrations of HPMC-K4 and carbopol 940. The tablets containing HPMC-K4M and carbopol-940 at 2:1 ratio (F11) showed satisfactory gastro retentive profile in terms of buoyancy lag time (60 sec), in vitro buoyancy time > 16 h and zero order drug release up to 6 h. In-vivo radiographic imaging technique confirmed that optimized formulation (F11) remained buoyant (4h) under physiologic conditions which is likely to produce a sustained drug release in the gastric fluid. In view of above results the formulation promises its utility for treatment of peptic ulcers in higher animals.

REFERENCES

- Arora S, Ali J, Ahuja A, Khar RK, Baboota S. Floating drug delivery systems: a review. *Aaps PharmSciTech*. 2005;6(3):E372-E390.
- Bahri-Najafi R, Mostafavi A, Tavakoli N, Taymouri S, Shahraki M-M. Preparation and in vitro-in vivo evaluation of acyclovir floating tablets. *Res Pharm Sci*. 2017;12(2):128.
- Bansal S, Beg S, Garg B, Asthana A, Asthana GS, Singh B. QbD-oriented development and characterization of effervescent floating-bioadhesive tablets of cefuroxime axetil. *AAPS Pharm Sci Tech*. 2016;17(5):1086-1099.
- Bettini R, Colombo P, Massimo G, Catellani PL, Vitali T. Swelling and drug release in hydrogel matrices: polymer viscosity and matrix porosity effects. *Eur J Pharm Sci*. 1994;2(3):213-219.
- Bhardwaj L, Sharma PK, Malviya R. A short review on gastro retentive formulations for stomach specific drug delivery: special emphasis on floating in situ gel systems. *Afr J Basic Appl Sci*. 2011;3(6):300-312.
- Blondeau J, Laskowski R, Bjarnason J, Stewart C. Comparative in vitro activity of gatifloxacin, grepafloxacin, levofloxacin, moxifloxacin and trovafloxacin against 4151 Gram-negative and Gram-positive organisms. *Int J Antimicrob Agents*. 2000;14(1):45-50.
- Diós P, Szigeti K, Budán F, Pócsik M, Veres DS, Máthé D, et al. Influence of barium sulfate X-ray imaging contrast material on properties of floating drug delivery tablets. *Eur J Pharm Sci*. 2016;95:46-53.
- Dudhipala N, Narala A, Janga K, Bomma R. Amoxicillin trihydrate floating-bioadhesive drug delivery system for eradication of *Helicobacter pylori*: Preparation, in vitro and ex vivo evaluation. *J Bioequiv Availab*. 2016;8(3):118-124.
- El-Zahaby SA, Kassem AA, El-Kamel AH. Design and evaluation of gastroretentive levofloxacin floating mini-tablets-in-capsule system for eradication of *Helicobacter pylori*. *Saudi Pharm J*. 2014a;22(6):570-579.
- El-Zahaby SA, Kassem AA, El-Kamel AH. Formulation and in vitro evaluation of size expanding gastro-retentive systems of levofloxacin hemihydrate. *Int J Pharm*. 2014b;464(1-2):10-18.
- Essa EA, Elkotb FE, Eldin EEZ, El Maghraby GM. Development and evaluation of glibenclamide floating tablet with optimum release. *J Drug Delivery Sci Technol*. 2015;27:28-36.
- Gharti K, Budhathoki U, Thapa P, Bhargava A. Formulation in vitro evaluation of floating tablets of hydroxypropyl methylcellulose and polyethylene oxide using ranitidine hydrochloride as a model drug. *J Young Pharm*. 2012;4(4):201-208.
- Gorman EM, Samas B, Munson EJ. Understanding the dehydration of levofloxacin hemihydrate. *J Pharm Sci*. 2012;101(9):3319-3330.
- Hadjiioannou TP, Christian GD, Koupparis MA, Macheras PE. Quantitative calculations in pharmaceutical practice and research: VCH New York; 1993.
- Higuchi T. Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. *J Pharm Sci*. 1963;52(12):1145-1149.
- Kshirsagar R, Jain V, Wattamwar S. Effect of different viscosity grade HPMC polymers on gastroretentive drug delivery of Metformin HCl. *Int J Appl Pharm*. 2009;1:44-50.
- Panda B, Subhadarsini R, Mallick S. Biointerfacial phenomena of amlodipine buccomucosal tablets of HPMC matrix system containing polyacrylate polymer/ β -cyclodextrin: correlation of swelling and drug delivery performance. *Expert Opin Drug Delivery*. 2016;13(5):633-643.
- Patel A, Modasiya M, Shah D, Patel V. Development and in vivo floating behavior of verapamil HCl intragastric floating tablets. *AAPS PharmSciTech*. 2009;10(1):310-315.
- Patel V, Patel N, Yeole P. Studies on formulation and evaluation of ranitidine floating tablets. *Indian J Pharm Sci*. 2005;67(6):703.
- Patel VF, Patel NM. Intragastric floating drug delivery system of cefuroxime axetil: in vitro evaluation. *AAPS PharmSciTech*. 2006;7(1):E118-E124.
- Patil H, Tiwari RV, Repka MA. Recent advancements in mucoadhesive floating drug delivery systems: A mini-review. *J Drug Delivery Sci Technol*. 2016;31:65-71.

Patil J. Design and evaluation of gastro retentive drug delivery system of anti ulcer drug. J Food Pharm Sci. 2015;3(1).

Porwal A, Dwivedi H, Pathak K. Decades of research in drug targeting using gastroretentive drug delivery systems for antihypertensive therapy. Braz J Pharm Sci. 2017;53(3).

Raghavendra Rao NG, Ram P, Kethan T, Suryakar V. Formulation and *in vitro* evaluation of gastric oral floating tablets of cefixime for controlled release. Res J Pharm, Biol Chem Sci. 2010;1(3)141-154.

Singla AK, Chawla M, Singh A. Potential applications of carbomer in oral mucoadhesive controlled drug delivery system: a review. Drug Dev Ind Pharm. 2000;26(9):913-924.

Vemula SK, Venisetty RK, Veerareddy PR. Valsartan floating bioadhesive compression-coated mini-tablets: Formulation and pharmacokinetics. J Drug Delivery Sci Technol. 2017;40:66-72.

Venkateswarlu K. Formulation Development and In-vitro Evaluation of Floating Tablets of Ciprofloxacin HCl. Asian Journal of Pharmaceutics (AJP): Free full text articles from Asian J Pharm. 2016;10(04).

Verma A, Dubey J, Hegde RR, Rastogi V, Pandit J. Helicobacter pylori: past, current and future treatment strategies with gastroretentive drug delivery systems. J Drug Target. 2016;24(10):897-915.

Wong PS, Dong L-C, Edgren DE, Theeuwes F, Gardner PI, Jao F, et al. Prolonged release active agent dosage form adapted for gastric retention. Google Patents. 2000.

Zaini E, Fitriani L, Effendy S, Noviza D, Halim A. Preparation and Characterization of Solid Dispersion Telmisartan–Hydroxypropyl Methyl Cellulose (HPMC) E5 LV by Co-Grinding Method. Orient J Chem. 2017;33(2):873-8.

Received for publication on 09th September 2018

Accepted for publication on 03rd July 2019