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Assessment of Antimicrobial activity of Moxifloxacin.HCl from Gastro Retentive Formulations

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ABSTRACT

Purpose: The objective of the present study is to assess the antimicrobial activity of gastroretentive formulation for moxifloxacin HCl and *in vitro* antimicrobial evaluation using the cup-plate method. **Materials and Methods:** Gastroretentive floating tablets of moxifloxacin HCl were prepared using variable amounts of hydroxypropyl methylcellulose (HPMC) K100M and *Lannea coromandelica* gum (LCG) by direct compression technique. **Results and Discussion:** Formulations were developed, optimized, and are checked for pharmacopoeial tests. Results show that all batches were lain within the standard limits. Further, *in vitro* drug release was assessed formulation (FS_5) by antimicrobial assay using *Staphylococcus aureus* and *Escherichia coli* as test microorganisms. Results reveal that released drug effective against both organisms for 24 h represents good antimicrobial activity. The minimum inhibitory concentration was founded to be 10 µg/mL. **Conclusion:** Formulation (FS_5) containing 50 mg of HPMC K100M and 50 mg of LCG expected to improve patient compliance by means of providing a good clinical outcome.

Keywords: Antimicrobial, gastroretentive, hydroxypropyl methylcellulose K100M, *in vitro*, *Lannea coromandelica* gum, moxifloxacin HCl

INTRODUCTION

The design of oral controlled drug delivery systems (DDS) is aimed to obtain desirable and enhanced bioavailability. Gastric emptying is a dynamic process and gastroretentivity of dosage form results improved clinical response.

Gastric transit time in humans, influences absorption of drugs, results alteration in clinical response. Gastroretentive DDSs retain the dosage form in the acidic environment for a longer period of time than conventional formulations. These systems are suitable for local drug delivery to the stomach and small intestine.[1] In case of the many drugs which are released within the stomach have the best therapeutic effect. These systems also had the benefit of less side effects and removed the need of repeated dosages.[2]

The controlled gastric retention of solid dosage forms was obtained by numerous mechanisms such as flotation, bioadhesion, high density (sedimentation), modified shape systems, expansion, or by simultaneous administration of pharmacological agents that delay gastric emptying.[3-5]

Floating DDS (FDDS) is also known as a hydrodynamically balanced system (HBS). FDDSs have a bulk density that is lower than gastric fluids and thus remain buoyant in the gastric environment for a prolonged period of time, without affecting the gastric emptying rate. The dosage form is stayed in stomach due to flotation mechanism, which results controlled rate of drug release. After the release of drug, the residual system is run out from the gastroenvironment; this will increase GRT and better control of fluctuations in plasma drug concentrations.[6-8] They also offer maintenance of C_{ss} longer period of time and minimizing the risk of resistance; this is very useful for the delivery of antibiotics.[9-10]

Moxifloxacin HCl, synthetic broad-spectrum antibacterial agent, belongs to the class of fourth-generation fluoroquinolone. It has a narrow absorption window and absorbed primarily in the proximal portions of gut, an ideal candidate for a gastroretentive drug delivery system that

will prolong the gastric transit time of formulation results enhanced bioavailability.[11,12]

An attempt is made in the present study to develop gastroretentive DDS (preferably by flotation) with the help of drug release rate modifiers (natural – *Lannea coromandelica* gum [LCG] and semisynthetic – hydroxypropyl methylcellulose [HPMC] K100M) and effervescent mixtures.[13-16] From the literature, very less work reported for LCG, though it is natural more benefits observed from an economy point of view as well as risk incidence also low. Hence, LCG selected as a polymer for the formulation development of moxifloxacin HCl gastroretentive delivery.

Most of the tablets were manufactured by utilizing the direct compression technique. However, drug release retardation also influenced by the method of manufacture. The literature survey proved that gastroretentive formulations were prepared by wet granulation technique also for bioadhesive delivery.[5]

The development of gastroretentive DDS of moxifloxacin HCl using polymers, which increases the gastric transit time, improves penetrability of the drug through the mucosa, thereby improving the clinical efficacy of the active ingredient.

MATERIALS AND METHODS

Materials

A gift sample of moxifloxacin HCl was procured from Macleods Pharmaceuticals Ltd., Mumbai, India. HPMCK100M was obtained from Loba Chemie Pvt. Ltd., Mumbai, India. LCG was gifted from Sarada Pharmaceuticals, Guntur. All other excipients such as sodium bicarbonate, lactose, Emcompress, magnesium stearate, and talc were obtained from S.D. Fine Chem. Ltd., Mumbai, India.

Formulation Development of Moxifloxacin HCl Gastroretentive Floating Tablets

Preparation of moxifloxacin HCl floating tablets (GRSOF)

The direct compression technique was utilized for the preparation of floating tablets, each containing 400 mg moxifloxacin HCl. Accurately weighed ingredients (except moxifloxacin HCl) were screened (# 40 mesh) for obtaining uniform size to ensure proper mixing and to obtain polymer mixture. The drug was then mixed with the polymer mixture for 10 min for uniform mixing of the powder blend. The blend was lubricated with magnesium stearate. The formulae for moxifloxacin HCl floating tablets are shown in Table 1. The powder blend was subjected to pre-formulation analysis. Results show good flow properties. The powder blend was subjected to compression with the help of a rotary tablet compression machine (tablet Minipress).

Compressed tablets were examined as per official standards and unofficial tests. Tablets were packaged in well-closed light resistance and moisture-proof containers.

Evaluation of Moxifloxacin HCl Gastroretentive Tablets[17]

Hardness

The breaking/crushing strength of the tablets was determined by measuring the diametric breakdown of tablets using a Monsanto tablet hardness tester.

Friability

The friability of the tablets was carried with the help of Roche friabilator. Twenty tablets were weighed noted as initial weight (W_0), these were subjected to 100 free falls from a fixed height and weighed (W) again. Percentage friability was calculated using the following formula. The friability result should not be more than 1%.

$$\text{Weight loss (\%)} = [W_0 - W/W_0] \times 100$$

Assay

The assay was performed by the triturating stated number of tablets in Indian pharmacopeia (20) converted to powder, powder equivalent to 100 mg of drug was added in 100 ml of 0.1 N HCl followed by sonication. The solution was filtered through a 0.45 µ membrane filter, suitable aliquots were prepared, and the absorbance of the resultant solution was measured spectrophotometrically at 288 nm using 0.1 N HCl as blank.[10,18]

Thickness

Thickness formulations were determined using Vernier calipers, by placing a tablet between two arms it.

In vitro buoyancy studies

This test is performed by placing the tablets in a beaker containing 100 mL of 0.1 N HCl (SGF). The time required

Table 1: Formulae for the preparation of moxifloxacin HCl floating tablets (DC)

Name of ingredients	Quantity of ingredients per each tablet (mg)								
	FS_1	FS_2	FS_3	FS_4	FS_5	FS_6	FS_7	FS_8	FS_9
Moxifloxacin HCl	436.8	436.8	436.8	436.8	436.8	436.8	436.8	436.8	436.8
Emcompress	17.2	32.2	47.2	32.2	47.2	62.2	47.2	62.2	77.2
HPMCK100M	65	65	65	50	50	50	35	35	35
Lannea coromandelica gum	65	50	35	65	50	35	65	50	35
Sodium bicarbonate	50	50	50	50	50	50	50	50	50
Magnesium stearate	3	3	3	3	3	3	3	3	3
Talc	3	3	3	3	3	3	3	3	3
Total weight	640	640	640	640	640	640	640	640	640

for the upward movement of tablet to float on the 0.1 N HCl (SGF) was noted to be floating lag time.[10]

In vitro drug release study

The *in vitro* dissolution rate study for formulation trails was performed using the USP XXIII Type II dissolution test apparatus containing 900 ml of 0.1 N HCl operated under conditions such as temperature 37 ± 0.5°C and rotated at a speed of 50 rpm. At predetermined time intervals, 5 ml of the samples were withdrawn as per the pharmacopoeial procedure. The resultant samples were analyzed for the estimation of drug release by measuring the absorbance at 288 nm using a UV–visible spectrophotometer after suitable aliquots. The samplings were performed in triplicate manner (n = 3).[5,10,11]

The dissolution profile of all the formulations was subjected to kinetic modeling such as zero-order, first-order, Higuchi, and Korsmeyer–Peppas models to know the drug release mechanisms.[19-22]

Swelling index study

To evaluate the swelling index, the tablet was placed in USP dissolution apparatus II with 900 ml 0.1 N HCl after measuring the weight of tablet (W_1). Then, the weight of tablet (W_2) was determined by virtue of time, i.e., at different time intervals, namely, 0, 2, 4, 6, 8, 10, and 12 h after using blotting paper to remove the surplus fluid. The swelling index was calculated using the following formula:

$$\text{Swelling index (\%)} = [(W_2 - W_1)/(W_1)] \times 100$$

Assessment of antibacterial activity

The tablet formulations which showed optimum drug release (FS_5) were taken for further evaluation using antimicrobial activity. The antimicrobial assay was carried out using the cup-plate method (agar diffusion). The different dilutions of standard were prepared in distilled water with concentrations ranging from 0 to 200 μg/mL. The aliquots obtained after dissolution were filtered through 0.45 μm nylon filter. Each 1 mL of the filtered samples was carefully transferred into the wells prepared with sterile cork borer on a solidified nutrient agar plate in Petri dishes inoculated with test Gram-positive cocci, *Staphylococcus aureus*, and Gram-negative bacilli, *Escherichia coli*. After inoculation, Petri dishes were kept in an incubator at 37°C for 24 h. After incubation, the diameter of the inhibition zone produced by tablet and standard dilution of antibiotic was measured with the help antibiotic zone reader in mm.[23]

Stability studies

An ideal controlled release dosage form should provide consistency of drug release throughout its shelf life. In the present investigation stability, studies were performed on the optimized formulation (FS_5). In each case, all formulations were packed in HDPE screw-capped bottles and kept in humidity chambers maintained at 25 ± 2°C, 60 ± 5% RH, 30 ± 2°C, 60 ± 5% RH, and 40 ± 2°C, 75 ± 5% RH as per the stability protocol of ICH guidelines.[24,25]

RESULTS AND DISCUSSION

Gastroretentive tablets of moxifloxacin HCl were formulated with the help of various drug release modifiers (HPMC K100M and LCG) along with effervescent mixtures. The formulation design is presented in Table 1. Powder blends were subjected to flow analysis. The results are summarized in Table 2. Pre-formulation results reveal that all formulations are passed. The limits and blends show good flow properties.

All trials have 436.8 mg of moxifloxacin HCl (equivalent to 400 mg of moxifloxacin) as a gastroretentive tablet dosage form prepared by the direct compression technique. All final batches were subjected to finished product performance evaluation tests such as drug content, floating lag time, adhesion time, mean hardness, total floating time, mean thickness, and friability as per pharmacopoeial methods, and subjective results are summarized in Table 3. From the results for post-compression tests, it was found that all batches were lain within the acceptance criterion. The purpose of the swelling study is to determine the water uptake capability of the retardant. The swelling study was performed on all formulation trials about 12 h. From the swelling study, it is found that all formulation trails were shown swelling phenomenon when come in contact with 0.1 N HCl but stayed without breaking during the study period. Formulation FS_5 was found to have the highest swelling property and the data for swelling evaluation are presented in Table 4 and Figure 1.

Drug release studies were performed for finished batches using pH 1.2 buffer (0.1 N HCl) as a dissolution fluid as operated under a standard set of conditions at 50 rpm (paddle), 37 ± 0.5°C. Comparative *in vitro* dissolution plots

Table 2: Pre-formulation studies for gastroretentive floating formulations

Formulation code	Angle of repose (°)	Compressibility index (%)	Hausner's ratio
FS_1	25.155±0.7	16.95±0.3	25.155±0.7
FS_2	25.855±0.6	16.725±0.2	25.855±0.6
FS_3	26.34±1.1	17.205±0.2	26.34±1.1
FS_4	25.1±0.7	16.455±0.3	25.1±0.7
FS_5	26.285±0.6	16.23±0.2	26.285±0.6
FS_6	26.285±1.1	16.71±0.2	26.285±1.1
FS_7	25.815±0.7	16.675±0.3	25.815±0.7
FS_8	26.2±0.6	16.05±0.2	26.2±0.6
FS_9	26.2±1.1	16.53±0.2	26.2±1.1
Moxifloxacin HCl	35.21±0.6	28.05±0.15	1.24±0.36

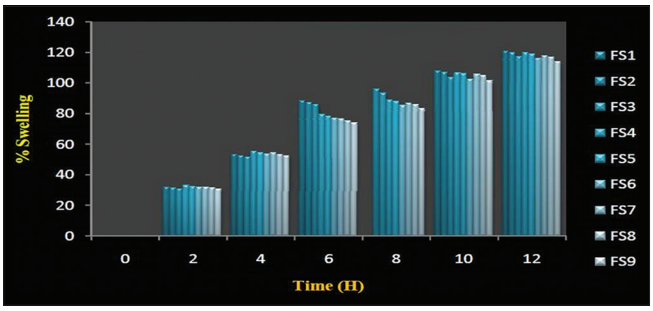

Figure 1: Percentage swelling with respect to time chart

Table 3: Post-compression parameters for floating formulations (n=3)

Formulation code	Hardness (kg/cm²)	Thickness (mm)	Friability (%)	Average weight (mg)	Drug content (%)	Floating lag time (s)	Total floating time (H)
FS_1	5.39±0.198	6.31±0.04	0.195±0.125	641.61±2.05	97.37±0.31	48.25±1.35	24
FS_2	5.19±0.188	6.26±0.02	0.345±0.085	643.62±4.05	97.715±0.36	50.86±1.4	24
FS_3	5.24±0.193	6.25±0.01	0.235±0.12	640.61±3.05	97.26±0.38	52.84±1.5	24
FS_4	5.46±0.18	6.24±0.04	0.18±0.13	642.5±2.15	98.96±0.33	49.34±1.4	24
FS_5	5.263±0.17	6.18±0.03	0.33±0.09	644.5±4.15	99.3±0.39	51.95±1.45	24
FS_6	5.32±0.18	6.18±0.01	0.22±0.125	641.5±3.15	99.95±0.41	53.93±1.55	24
FS_7	5.68±0.22	6.27±0.04	0.175±0.115	641.61±2.05	99.91±0.43	51.86±1.5	24
FS_8	5.48±0.21	6.22±0.02	0.325±0.075	643.62±4.05	99.26±0.49	54.47±1.55	24
FS_9	5.54±0.21	6.21±0.01	0.215±0.11	640.6±3.05	100.6±0.51	56.45±1.65	24

Table 4: Swelling index of moxifloxacin HCl floating formulations

Formulation code	Percentage swelling with respect to time (h)					
	2	4	6	8	10	12
FS_1	31.97±0.1	53.4±0.2	88.49±0.3	96.0.38±0.3	107.7±0.4	120.83±0.4
FS_2	31.43±0.3	52.4±0.4	87.28±0.2	95.9±0.5	106.95±0.4	119.92±0.3
FS_3	30.9±0.4	51.47±0.4	85.95±0.2	93.23±0.4	103.5±0.3	117.15±0.3
FS_4	32.97±0.2	55.4±0.1	79.49±0.2	88.8±0.2	106.7±0.3	119.83±0.2
FS_5	32.43±0.3	54.4±0.4	78.28±0.5	87.9±0.4	105.95±0.3	118.92±0.4
FS_6	31.9±0.3	53.47±0.5	76.95±0.6	85.23±0.4	102.5±0.3	116.15±0.3
FS_7	31.97±0.2	54.4±0.1	76.49±0.2	86.8±0.2	105.7±0.3	117.83±0.2
FS_8	31.43±0.3	53.4±0.4	75.28±0.5	85.9±0.4	104.95±0.3	116.92±0.4
FS_9	30.9±0.3	52.47±0.5	73.95±0.6	83.23±0.4	101.5±0.3	114.15±0.3

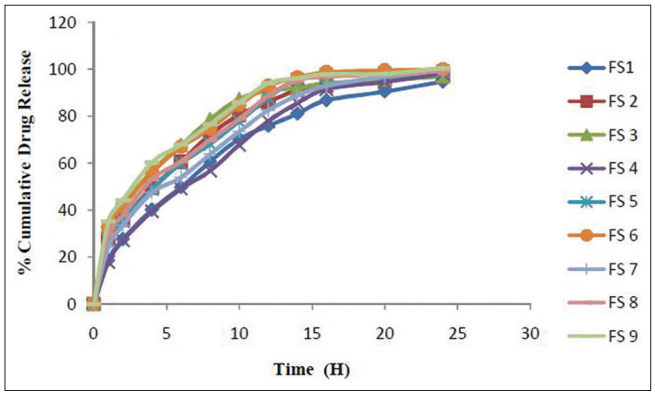

Figure 2: *In vitro* dissolution profiles for FS_1–FS_9

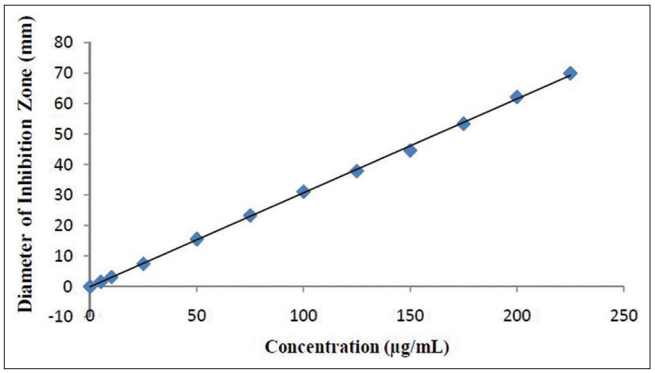

Figure 3: Calibration curve against *Staphylococcus aureus*

are presented in Figure 2. Percentage cumulative drug release (CDR) for finished batches F_1–F_9 at 24 h was found to be 99.25 ± 0.66–99.31 ± 2.1%. The result revealed that the release rate of drug was inversely proportional to the quantity of polymers and vice versa.[26] Hence, the desired drug release was achieved by manipulating the composition of independent variables.

The formulation FS_5 showed optimum drug release properties, hence considered as the best formulation among all batches. This formulation was subjected to antimicrobial assessment using a cup-plate method along with standard concentrations of antibiotic against *S. aureus* and *E. coli* as test organisms. Calibration curves, antimicrobial susceptibility profiles are presented in Figures 3-5 respectively. From the results, it was observed that zones of inhibition for both the test organisms were nearly the same. The FS_5 exhibited optimum inhibition, the diameter of inhibition zone against *S. aureus* and *E. coli* was founded to be 68.58 ± 0.66 and 67.39 ± 0.63, respectively, at the end of 24 h dissolution sample. Formulation FS_5 further subjected to stability analysis as per the ICH guidelines.

No visible physical changes were observed in the FS_5 withdrawn from the humidity chambers. The hardness, moisture content, and drug content in all the formulations

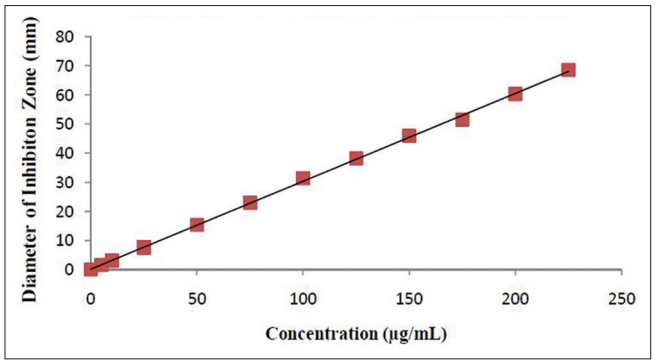

Figure 4: Calibration curve against *Escherichia coli*

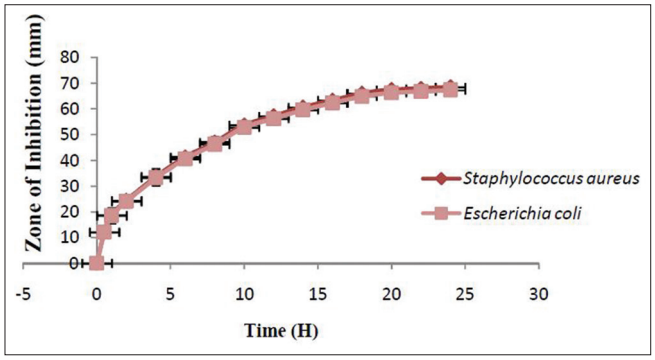

Figure 5: Antimicrobial susceptibility profile against *Staphylococcus aureus* and *Escherichia coli*

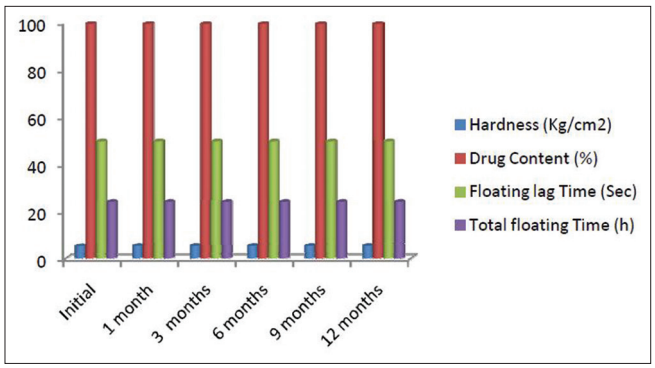

Figure 6: Stability data for FS_5 at 25 ± 2°C 60 ± 5% RH for 1 year (LST)

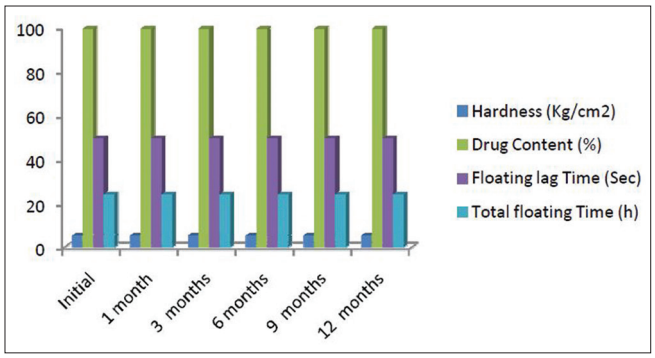

Figure 7: Stability data for FS_5 at 30 ± 2°C 65 ± 5% RH for 1 year (IST)

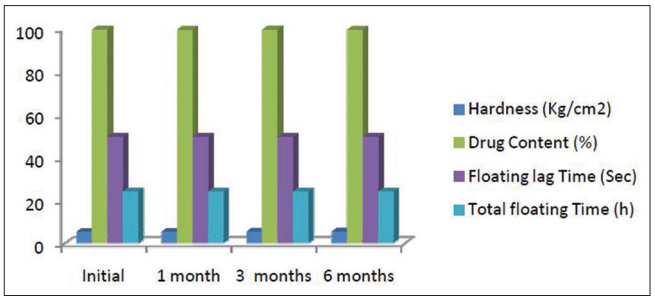

Figure 8: Stability data for FS_5 at 40 ± 2°C 75 ± 5% RH for 6 months (AST)

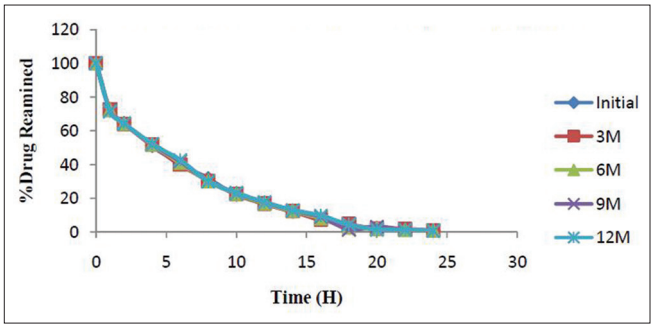

Figure 9: Percentage drug remained for FS_5 at 25 ± 2ºC 60 ± 5%RH for 1 year (LST)

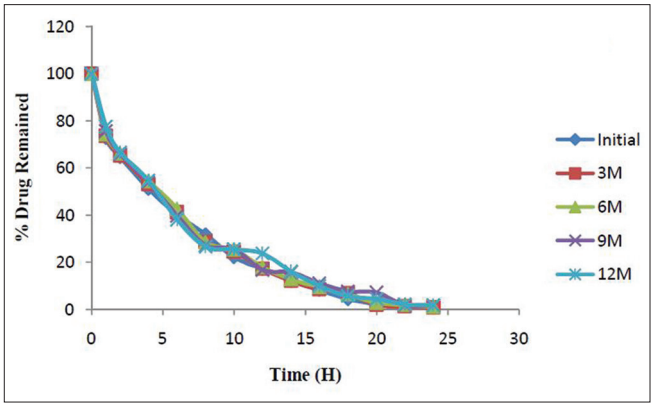

Figure 10: Percentage drug remained for FS_5 at 30 ± 2°C 65 ± 5% RH for 1 year (IST)

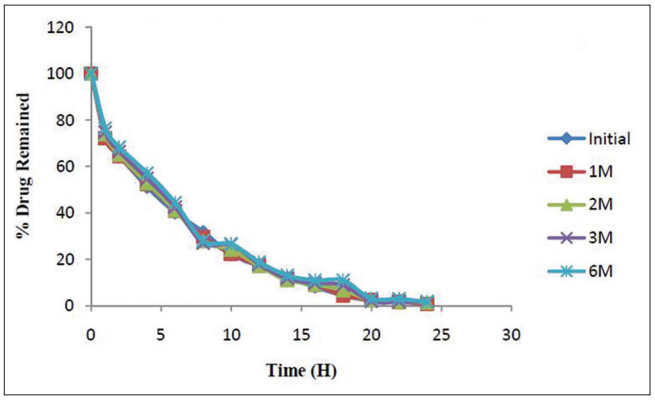

Figure 11: Percentage drug remained for FS_5 at 40 ± 2°C 75 ± 5% RH for 6 months (AST)

were found to be satisfactory. The release profiles of all the formulations have not changed significantly after storage at 25 ± 2°C, 60 ± 5% RH, 30 ± 2°C, 60 ± 5% RH for 12 months, and 40 ± 2°C, 75 ± 5% RH for 6 months. The slow and controlled drug delivery of the moxifloxacin remained constant. Results for finished product assurance tests are summarized in Figures 6–8. Comparative percentage drug remained versus time plots at different conditions are shown in Figures 9-11.

CONCLUSION

On the basis of the present research study, the use of macromolecules (natural and semisynthetic polymers) in combination had its own advantages of maintaining integrity and buoyancy of tablets. Gastroretentive tablet dosage form of moxifloxacin HCl was formulated successfully using HPMC K100M and LCG. The effervescent-based FDDS is a promising formulation to obtain gastroretentivity using gel-forming polymers employing sodium bicarbonate as gas generating agent. Among the various gastroretentive formulations studied, the formulation FS_5 showed the best result in terms of the required % CDR, floating lag time, and total floating time and is considered as the ideal formulation. Best formulation F_5 follows first-order release and non-Fickian diffusion. It shows good retaining characteristics. The *in vitro* antimicrobial activity also maintained well. Results for stability studies of FS_5 found to be satisfactory. It also avoids first-pass effect and also improves patient compliance by reducing the dosing frequency, which will ultimately improve the clinical outcome.

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