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In Vivo floating behaviour Assessment of Levofloxacin Floating Tablets Prepared via Experimental Design Approach for *Helicobacter pylori* Infection

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Abstract

The purpose of this study was to evaluate the in vivo floating behaviour of levofloxacin floating tablets that had been produced using an experimental design approach for the purpose of treating an infection caused by *Helicobacter pylori*. A three-factor, three-level Box-Behnken design was employed in the formulation of the floating tablets. The, HPMC K100M content, and HPMC K4M content and the Xanthan gum content were employed as the independent variables in this model. The optimized formulation exhibited desirable *in vitro* characteristics, including a high swelling index and prolonged drug release. In the *in vivo* investigation using rabbits, X-ray imaging demonstrated the gastric retention of the levofloxacin floating tablets over a period exceeding 12 hours. The tablets remained buoyant in the upper portion of the stomach, indicating their sustained floating behaviour. These findings highlight the potential of the developed levofloxacin floating tablets as an effective drug delivery system for *H. pylori* eradication, offering prolonged gastric retention and improved therapeutic outcomes.

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INTRODUCTION

Helicobacter pylori, often known as *H. pylori*, is a type of gram-negative bacterium that lives in the stomachs of roughly half of the people in the world [1]. An infection with *H. pylori* is a significant risk factor for a variety of gastroduodenal disorders, including gastric cancer, gastroduodenal ulcer disease, and a variety of other gastric and extra-gastric diseases [1,2]. The eradication of this pathogen, which is responsible for up to 95% of duodenal ulcers and up to 85% of stomach ulcers, significantly decreases the risk of ulcer recurrence [1]. *H. pylori* has been classified as a "category 1" pathogen by the International Agency for Research on Cancer (IARC), which indicates that it is certainly a cancer-causing agent [1,3]. In addition, the World Health Organisation (WHO) suggests getting rid of *H. pylori* to reduce the likelihood of developing stomach cancer [1]. There have been multiple studies that have demonstrated that a successful eradication of H. pylori substantially lowers the chance of developing stomach cancer [1,4]. In spite of this, achieving a complete and effective eradication of *H. pylori* has grown into an increasingly difficult task over the course of the last few years. [1]. Studies [5, 6] employing biopsy and cell culture infection models have demonstrated that *Helicobacter pylori* (*H. pylori*) is able to enter the gastric mucus layer and attach itself to various phospholipids and glycolipids that are included within the mucus gel. [1] These findings support the hypothesis that *H. pylori* causes gastric ulcers. As a consequence of this, the lumen of the stomach as well as the blood flow to the stomach can limit the amount of antibiotics that are available in the mucus layer for an extended period of time. In addition, traditional ways of administering drugs are unable to remain in the stomach for an adequate amount of time and do not successfully transport fully active antibiotics to the site of an infection [1].

Because of this, developing of innovative drugs delivery systems is an absolute necessity in order to overcome the restrictions that occur with current delivery methods. Floating drug delivery systems are able to remain float in the stomach due to their reduced bulk density as compared to the fluids found in the stomach. This allows for longer drug delivery that is unaffected by the rate at which the stomach empties. Levofloxacin Floating Tablets were prepared by our research team using an Experimental Design Approach since they were aware of the benefits that floating tablets bring to the table when it comes to the treatment of Helicobacter pylori infection. The process of optimisation was carried out with the assistance of Design-Expert® 13 software (Stat-Ease, Inc., United States), which was used to support a three-factor, three-level Box-Behnken design. In this particular study, the amounts of HPMC K4M (A1) materials, HPMC K100M (B2) material, and Xanthan gum (C3) materials were the independent variables that were explored. After completing an adequate number of preliminary tests, the amounts for these three components were figured out. The swelling index (SI), the floating lag time (FLT), and the amount of time needed for 90% of the drug to be released from the tablet were chosen to be the dependent variables that would be evaluated. The different formulations were subjected to in vitro testing, and the results of that testing were published (7). The formulation that demonstrated the best qualities when tested in vitro was chosen for further investigation in vivo. The objective of this body of work is to explore the floating behaviour of the selected formulation when it is in vivo (7). The in vivo investigation will help in the development of efficient medication delivery methods for Helicobacter pylori infection by providing useful insights into

the floating behaviour of the selected formulation.

MATERIAL AND METHODS

Preparation of Floating Tablets of levofloxacin Tablets

Levofloxacin were gifted by MICRO LABS LIMITED, Bengaluru, Xanthan gum was purchased from SD Fine Chem Limited, Mumbai. HPMC K100M and HPMC K4M, All other used solvents were HPLC grade. Table 1 outlines the experimental design and corresponding formulations. In order to effectively evaluate a 3-factor, three-level Box-Behnken statistical experimental design, the RSM requires 15 independent experiments, In Table 2, the independent variables and the *invitro* results of of the experiment listed. Direct compression was used as the method of preparation for the levofloxacin floating.

After preparing Floating Tablets of levofloxacin according to the method described earlier using the afore mentioned drug and excipients, *in vitro* studies were conducted and reported (7). The formulation with the best *in vitro* characteristics, determined from the *in vitro* data, F12 was chosen for this in *vivo* study (Table 3)

Table 1: Box-Behnken experimental design Layout .

Independent variable	**Levels**		
	-1	**0**	**1**
A_1- HPMC K4M(mg)	40	60	80
B_2-HPMC K100M(mg)	30	45	60
C_3-Xanthan gum(mg)	15	30	45
Dependent variables			
Y_{FLT} = Floating Lag Time (min)			
Y_{SI} = Swelling Index (%)			
$Y_{T90\%}$ = Time required to release 90% of the drug from the tablet ($T_{90\%}$)			

Table 2: Factorial batch formula -Levofloxacin floating tablets - Box–Behnken Design

Ingredients (mg)	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**	**F10**	**F11**	**F12**	**F13**	**F14**	**F15**
Levofloxacin	250	250	250	250	250	250	250	250	250	250	250	250	250	250	250
HPMC K4 M	60	80	40	80	80	40	60	60	60	60	60	60	40	40	80
HPMC K100 M	45	45	60	60	30	45	60	45	45	30	30	60	45	30	45
Xanthan gum	30	45	30	30	30	15	15	30	30	15	45	45	45	30	15
Sodium bicarbonate	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50
Citric acid	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
Microcrystalline Cellulose	40	5	45	5	35	75	40	40	40	70	40	10	45	75	35
Magnesium Stearate	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Total (mg)	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500

Table 3:Observed Responses - optimized batch F12 in the Box-Behnken design.

Formulation Code	Independent variables			Dependent variable
	A [mg]	B [mg]	C[mg]	$T_{90\,\%}$ [h]
F12	60	60	45	10.17±0.289

Mean ± SD, *n*=3, SD: Standard Deviation

Evaluation of Gastric Retention Using X-Ray Imaging (8)

An X-ray imaging investigation was conducted on rabbits (*n = 2*) to evaluate the stomach retention characteristics of the selected Floating Tablets of levofloxacin **(JKKN/IAEC/Ph.D/07/2021)** In order to make the tablets visible under X-ray, barium sulfate was added as an opaquing agent in the reformulated tablets for the in vivo investigation, while keeping the other ingredients constant. Prior to the experiment, the rabbits (*n = 2*) underwent a 12-hour fasting period. The optimized tablet was then administered to each rabbit orally by natural swallowing, followed by 30 mL of water. The rabbit had a wooden block with a hole in the middle placed in between its upper and lower teeth, and the tablet was given to the rabbit through the rabbit's mouth using an oral gavage procedure that involved a rubber bulb and a polyethylene tube . A additional flush of 5-10 mL of water was administered into the rabbit's mouth in order to guarantee that the dose form was completely delivered. For the purpose of establishing a baseline, X-ray photographs were acquired before to the administration of the tablet (during the pre-treatment phase). These images used to verify that the stomachs of the test subjects did not contain any barium sulfate-containing materials. Following the intake of the tablet, a skilled radiologist took X-ray pictures of the stomach region of the rabbit at various time intervals including 0 hours, 2 hours, 4 hours, 6 hours and 12 hours. Imaging with X-rays was carried out with the assistance of a Genius-60 Mobile portable equipment, which was manufactured by Wipro GE Medical Systems Ltd. in Pune, India. with parameters set at 40mA, 45KV, and 5mAs. The distance between the X-ray source and the rabbit's abdomen was maintained at a constant 80 cm for all images.

RESULTS AND DISCUSSION

The present study utilized an X-ray radio-opaque marker to capture instantaneous photographs of an optimized levofloxacin floating tablet, allowing for easy detection of its transition in the gastrointestinal tract (GIT) through X-ray imaging. Each X-ray image provided clear visibility of the highlighted organs, enabling individual interpretation. The investigation focused on assessing the gastric retention property of the optimized floating matrix tablet in rabbits. X-ray photographs were taken at different time intervals following the administration of the drug-free formulation to the rabbits.

The X-ray image clearly demonstrated the absence of the tablet in the rabbit's GIT. However, two hours after oral administration, an X-ray image of the stomach showed clear detection of the levofloxacin floating tablet in the upper portion of the stomach (Figure 1). Subsequent X-ray imaging at the 6th and 12^{th} hours indicated the presence of the tablet in the stomach region, albeit with a shifted location within the stomach area as depicted in Figure 1. This observation confirmed the gastric retention property of the tablet. The experiment revealed that the optimized levofloxacin floating tablet continuously floated in the stomach area of the rabbits, thus extending the gastric retention time beyond 12 hours.

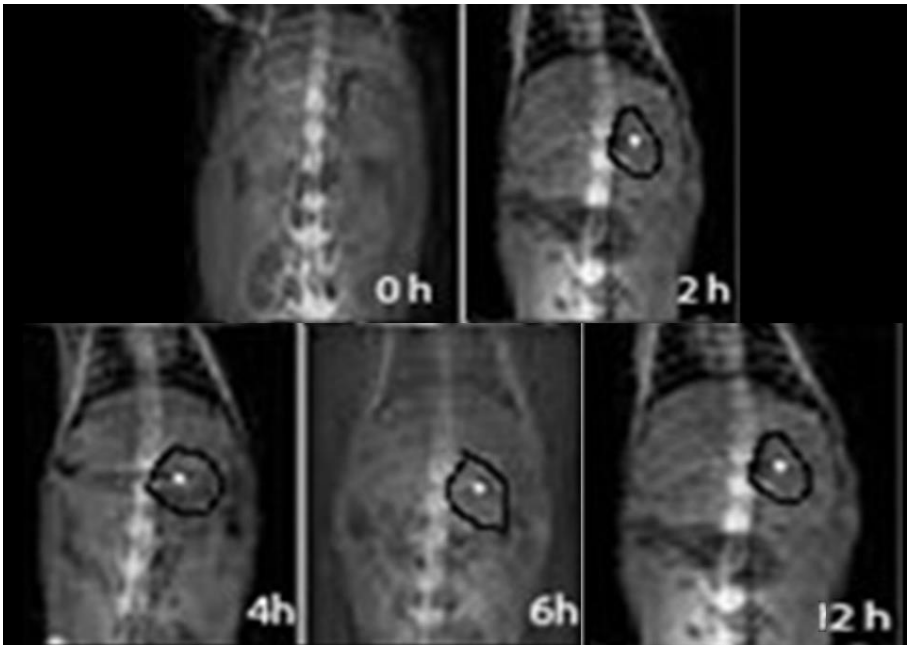

Figure 1: X-ray photographs of the $BaSO_4$-loaded Levofloxacin floating tablets (F12) in the stomach at (a) 0h, (b) 2h, (c) 4h, (d) 6h, and (e) 12h

CONCLUSION

In conclusion, this research article focused on the development and evaluation of floating tablets of levofloxacin for gastric retention and effective drug delivery. The study utilized an X-ray imaging investigation on rabbits to assess the stomach retention characteristics of the formulated tablets. By incorporating barium sulfate as an opaquing agent, the tablets were made visible under X-ray, enabling real-time monitoring of their movement within the gastrointestinal tract. The results of the *in vivo* study demonstrated the successful gastric retention of the optimized levofloxacin floating tablets. X-ray photographs captured at different time intervals provided clear evidence of the tablets' presence in the upper portion of the stomach, indicating their buoyancy and ability to remain in the gastric region. Furthermore, the tablets exhibited sustained floating behavior over a period exceeding 12 hours. These findings highlight the potential of floating tablets as a promising drug delivery system for levofloxacin for *H.pylori* therapy. The extended gastric retention time offered by the formulated tablets can enhance drug availability in mucus layer and improve therapeutic efficacy of levofloxacin for *H. pylori* infection.

The X-ray imaging investigation proved to be a valuable tool for monitoring the movement and behavior of the floating tablets *in vivo*. The use of barium sulfate as an opaquing agent provided clear visualization and facilitated the interpretation of X-ray images.

Overall, the successful development and evaluation of levofloxacin floating tablets with prolonged gastric retention properties provide a promising approach for improving drug delivery and treatment outcomes in H. pylori infection. Further research and clinical trials are warranted to assess the safety, efficacy, and pharmacokinetic profile of these floating tablets in human subjects.

REFERENCES

1.Malfertheiner P, Megraud F, O'Morain CA, Atherton J, Axon AT, Bazzoli F, et al. Management of Helicobacter pylori infection—the Maastricht V/Florence Consensus Report. Gut. 2017 Jan 1;66(1):6-30.

2.Hooi JKY, Lai WY, Ng WK, Suen MMY, Underwood FE, Tanyingoh D, et al. Global prevalence of Helicobacter pylori infection: systematic review and meta-analysis. Gastroenterology. 2017 Aug 1;153(2):420-9.

3.International Agency for Research on Cancer. Schistosomes, liver flukes and Helicobacter pylori. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 7-14 June 1994. IARC Monogr Eval Carcinog Risks Hum. 1994;61:1-241.

4.Ford AC, Forman D, Bailey AG, Axon AT, Moayyedi P, Lévi F. Effect of eradication of Helicobacter pylori on incidence of metachronous gastric carcinoma after endoscopic resection of early gastric cancer: an open-label, randomised controlled trial. The Lancet. 2020 Nov 7;396(10251):1506-12.

5.El-Shoura SM. Helicobacter pylori: I. Ultrastructural sequences of adherence, attachment, and penetration into the gastric mucosa. Ultrastruct Pathol. 1995;19(4):323-33.

6. Engstrand L, Graham DY, Scheynius A, Genta RM, El-zaatari F. Is the sanctuary where helicobacter pylori avoids antibacterial treatment intracellular? Am J Clin Pathol. 1997;108(5):504-9.

7. Jaganathan K et al. Experimental design approach to fabricate and optimize floating tablets of levofloxacin for helicobacter pylori infection. Int J App Pharm.2022;14(6):100-13.

8. Sarangapani S. In vitro and in vivo evaluation of the gastro retentive floating dosage form. Int Res J Pharm.2014;5(9):695-700.