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Research Article

APPLICATION OF DESIGN OF EXPERIMENTS TO OPTIMIZE GASTRO RETENTIVE DRUG DELIVERY SYSTEMS FOR CIPROFLOXACIN HCL AND METRONIDAZOLE

Rohit Lowalekar*, Lalit Singh Chauhan

Department of Pharmacy, Mohan Lal Sukhadia University, Udaipur

Bhupal Nobels Institute of Pharmaceutical Sciences, Udaipur, Rajasthan

ABSTRACT

Ciprofloxacin HCl and metronidazole both have a short half-life and narrow absorption window. Moreover, metronidazole is ineffective on aerobic bacteria. Thus a combination of both can be effective on a broad spectrum of bacteria. The objective of this research was to develop gastroretentive floating tablets of Ciprofloxacin Hcl and metronidazole using 2^3 full factorial design with 3 factors at two levels. The factors responsible for design space were selected based on the primary screening of the formulation for different polymer matrix and floating agent guar gum. The statistically significant study resulted in eight experiments wherein the formulations were evaluated for hardness, floating time, friability, % drug content, in-vitro drug release and mean gastric retention period. Statistical analysis was done using Minitab 17.3.1 software along with data model fitting. One-way Analysis of variance (ANOVA) was carried out to determine the p-value, F-value, R^2 and the standard deviation (S) of the various factors over various responses individually at a confidence limit of 95%. Surface plots were plotted between a single pair of variables and the required response to be measured to relate to the two continuous variables. Statistical analysis data revealed that tablets from formulation batch F2 was more promising system exhibiting excellent floating properties and drug release pattern. The study and the statistical parameters depicted that there is no difference between group means on the entire study factors. Stability studies revealed that all formulations were physically and chemically stable.

KEYWORDS: *Ciprofloxacin HCL, Metronidazole, Factorial design, In-vitro drug release, ANOVA.*

INTRODUCTION

Drugs that are easily absorbed from gastrointestinal tract (GIT) and have short half-life are eliminated quickly from the systemic circulation. Frequent dosing of these drugs is required to achieve suitable therapeutic activity [1]. To formulate a site-specific orally administered controlled release dosage form, it is desirable to achieve a prolonged gastric residence time by the drug delivery. Prolonged gastric retention improves bioavailability, increases the duration of drug release, reduces drug waste, and improves the drug solubility that are less soluble in a high pH environment [2].

**Author for correspondence:-*
Rohit Lowalekar,
Bhupal Nobels, Institute of Pharmaceutical Sciences, Udaipur, Rajasthan,
E-mail id: rohit.lowalekar@gmail.com

Gastro-retentive drug delivery is an approach to prolong gastric residence time, thereby targeting site-specific drug release in the upper GIT for local or systemic effects. Gastro-retentive dosage forms can remain in the gastric region for long periods and hence significantly prolong the gastric retention time (GRT) of drugs. Over the last few decades, several gastro-retentive drug delivery approaches being designed and developed, including: high density (sinking) systems that are would come, not is retained in the bottom of the stomach [3], low density (floating) systems that causes buoyancy in gastric fluid [4-6], mucoadhesive systems that causes bioadhesion to stomach mucosa [7], unfoldable, extendible, or swellable systems which limits emptying of the dosage forms through the pyloric sphincter of stomach [8,

9], super-porous hydrogel systems [10], magnetic systems [11].

Medicines are mixtures of multiple components. The component proportions are critical to the effectiveness of the medicine. Thus the quality characteristic of medicine depends on the proportions of components. The mixture experiments are especially useful for solving the problem of searching the optimal proportions of the components [12]. In a factorial design the influences of all experimental variables, factors, and interaction effects on the response or responses are investigated. If the combinations of k factors are investigated at two levels, a factorial design will consist of 2k experiments [13].

Analysis of variance (ANOVA) is the most efficient method available for the analysis of experimental data. ANOVA is a method of considerable complexity and subtlety, with many different variations, each of which applies in a particular experimental context [14]. ANOVA is a statistical technique that identifies factors significantly affecting the experimental results and consists of p-value, F-value, R^2 and the standard deviation (S) [15].

Ciprofloxacin Hcl is a broad-spectrum fluoro-quinolone antibacterial agent that has most of its absorption from the stomach and the proximal part of the small intestine [16]. Thus , not this floating sustained release system is chosen for this drug primarily for this reason. It is freely soluble in water and its oral bioavailability is about 70% and reaches the peak plasma concentration of 2.5µg/ml in 1 to 2 h after administration of 500 mg equivalent but has a short half-life of 4 h. However, it shows a decrease in the absorption from the lower GIT. The drug is mostly used for indications like uncomplicated urinary tract infections (UTIs) [17, 18] and for bone and joint infections, infectious diarrhoea, lower respiratory tract infections, hospital-acquired infections and meningococcal prophylaxis [19].

Metronidazole & Ciprofloxacin Hcl are active against a broad spectrum of obligate anaerobic bacteria, including *Bacteroids* spp., *Fusobacterium* spp., *Clostridium* spp., *Treponema* spp. and various anaerobic cocci. The action is trichomonicidal, and bactericidal [20]. Further, they do not interfere with each other and thus provide a complete treatment of the aerobic and anaerobic bacteria together [21].

Optimization techniques have been applied in the present study to systemically study the influence of process variables on the formulation of dosage forms for selection and optimization of polymer concentration and optimization of guar gum quantity that has pronounced effect on tablet properties and drug release profile of the formulation. These designs provide an effective means for studying the effect of various parameters on the dependent variables with an objective to prepare and evaluate gastro-retentive floating tablet of metronidazole and Ciprofloxacin Hcl combination to increase the residence time in the stomach and there by gives prolonged action of each drug individually.

MATERIALS AND METHODS

Materials

Ciprofloxacin Hcl (Taj Pharmaceuticals Ltd., India), Metronidazole (Ciron drugs, India), hydroxypropyl methylcellulose (HPMC E10M and HPMC K100M), (Shin-Etsu Chemical Corporation, Japan), Guar gum (alpha chemicals, India), Polyvinyl pyrrolidone K-30 (Glide chem, India), Citric acid (Merck, Germany), sodium bicarbonate (Merck, Germany), Microcrystalline cellulose (Accent microcell Pvt. Ltd., India) magnesium stearate (S.D. Fine Chemical Pvt)

Methodology

A 2^3 full factorial design using Minitab 17.3.1 was employed with three factors at two different levels to optimize the concentration of the ratio of polymers HPMC K100M and guar gum respectively. The factors employed for the studies were concentration of HPMC K100M, concentration of HPMC E10M and concentration of guar gum. The responses studied against the factors were floating lag time and drug release profile of Ciprofloxacin Hcl and metronidazole. Eight experiments (F-1 to F-8) were designed with statistical significance with a lower level of 66.6mg of both polymers and 50mg of guar gum while higher level comprising of 133.3mg of both polymers and 100mg of guar gum

respectively. All ingredients (except glidants, lubricant and PVP K-30) were passed through sieve No. 30 and mixed thoroughly in planetory mixer at 200 rpm. To this mixture MCC PH101 previously passed through sieve No. 44 was added and further mixing was carried in the planetory mixer. After thorough mixing granulation was carried using 6.5% w/v solution of PVP-K30 in isopropyl alcohol in the planetory mixer. The wet mass was subjected to drying on fluidized bed drier of 1 kg capacity, for a period of one hour at 40 ºC. The dried granules were then sized by sieve No. 22 and magnesium stearate was added as extra granular to enhance flow property and lubrication. The granules thus obtained were compressed into tablets on a Cadmach 16-station, single rotatory, compression machine (D-tooling type). The powder blend for all the batches was studied for micromeritic properties and the formulated tablets were evaluated for hardness, friability, weight variation, drug content, floating lag time, floating duration and the drug dissolution for Ciprofloxacin Hcl and metronidazole using 0.1 N Hcl at 37± 0.5° C (900 ml using USP apparatus II at 50 rpm.). The compositions of different excipients in formulations (F1-F8) are listed in Table I. The polynomial equation used for this study can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries, i.e. positive or negative. The high values of correlation coefficient for the dependent variables indicate a good fit. The equation may be used to obtain estimate of the response because small error of variance was noticed in the replicates. The mathematical equation for a 2^3 full factorial design is as follows:

$$\mathbf{Y= \beta o + \beta_1 \cdot x_1 + \beta_2 \cdot x_2 + \beta_{12} \cdot x_1x_2 + \beta_3 \cdot x_3 + \beta_{13} \cdot x_1x_3 + \beta_{23} \cdot x_2x_3 + \beta_{123} \cdot x_1x_2x_3 + \varepsilon.} \quad \mathbf{(1)}$$

Where, βo represents the overall mean, β_1 represents the independent effect of the first factor A, β_2 represents the independent effect of the second factor B, β_{12} represents the independent effect of the interaction of factor A and B, β_3 represents the effect of the third factor C, β_{13} represents the effect of the interaction of factor A and C, β_{23} represents the effect of the interaction of factor B and C, β_{123} represents the effect of the interaction of factor A, B, and C and ε is the random error term. Surface plot were plotted between a single pair of variables and the required response to be measured. It showed how the response relates to the two continuous variables. After optimization of the polymer and guar gum concentration, one-way ANOVA was carried out between all the factors and the desired responses such as effect of concentration of HPMCK100M on floating lag time, % Ciprofloxacin Hcl and % metronidazole release. Similarly, the effect of concentration of HPMC E10M and guar gum concentrations were studied for the same responses to establish statistical significance for the design of experiments. For all the studies, p-value, F-value, R^2 and the standard deviation (S) of the various factors over various responses were studied at a confidence limit of 95%. S is measured in the units of the response variable and represents the standard deviation of how far the data values fall from the fitted values. The lower the value of S, the better the model describes the response. R^2 is the percentage of variation in the response that is explained by the model. The higher the R^2 value, the better the model fits your data. To determine whether any of the differences between the means are statistically significant, compare the p-value to your significance level to assess the null hypothesis.The optimized batch of gastro-retentive tablets of Ciprofloxacin Hcl and metronidazole were packaged using HDPE bottles for stability studies at 25 ºC / 60 % RH. These tablets were stored at 40 ºC / 75% RH for a period of 3 months. From the tablets so stored, samples were withdrawn at the end of each month and were evaluated for visual appearance, in vitro floating characteristics and drug content. The floating lag time and the drug release profile of Ciprofloxacin Hcl and metronidazole was compared to that of initial after every month.

Table 1: Design of batches F1 to F8 using various ratios of polymers HPMCK100M, HPMCE10M and guar gum using 2^3 full factorial design.

Ingredients (mg)	F1	F2	F3	F4	F5	F6	F7	F8
Ciprofloxacin Hcl	582.1	582.1	582.1	582.1	582.1	582.1	582.1	582.1
Metronidazole	500	500	500	500	500	500	500	500
HPMCK100M	66.6	133.3	66.6	133.3	66.6	133.3	66.6	133.3
HPMCE10M	66.6	66.6	133.3	133.3	66.6	66.6	133.3	133.3
Guar gum	50	50	50	50	100	100	100	100
PVP K 30	15	15	15	15	15	15	15	15
Citric Acid	20	20	20	20	20	20	20	20
NaHCO3	60	60	60	60	60	60	60	60
MCC	50	50	50	50	50	50	50	50
Mg. Stearate	10	10	10	10	10	10	10	10
Total wt.	1403.7	1487	1487	1553.7	1470.3	1537	1537	1603.7

RESULTS AND DISCUSSION

The micromeritic properties of powder blends of batches F1-F8 are depicted in table II. The angle of repose for all the batches depicts excellent flow property with values between 25-30°**.** The loose bulk density and tapped bulk density for the batches are comparable without any major variation. The Carr's index for F1, F2 and F6 shows good flow properties which is depicted by Carr's index below 17 whereas other batches shows fair flow property as the Carr's index is between 17-21%. Hausner's ratio of all the batches depicts good flow properties whereas, batches F3 and F8 shows bad flow property. The quality control tests for tablets are shown in table III. The hardness and friability of all the batches is well within limits of hardness test. All the batches passed the weight variation test. All the batches complies the assay range of 90-110 % both for Ciprofloxacin Hcl and metronidazole. The order of floating lag time for ratio of polymers is F2>F5>F3, F8>F6>F7>F1>F4 where F2 achieved the desired floating lag time of 75 s indicating F2 to be the ideal formulation as far as floating lag time is concerned. The drug release profile of Ciprofloxacin Hcl and metronidazole for batches F1-F8 and target profile Cifran OD and Flagyl ER are depicted in figure I and II respectively.

The order of drug release for Ciprofloxacin HCl is F2>F1>F7>F3>F6>F8>F5>F4 indicationg F2 having similar drug release profile as target profile of Cifran OD. The order of drug release for metronidazole is F2>F1>F7>F3>F6>F8>F5>F4 indicating F2 having similar drug release profile as target profile of Flagyl ER. All of the F2 Values are above 50 which represent a similar dissolution profile, while that of F2 is nearing 100 indicating an identical dissolution profile as compared to the reference drug Cifran OD and Flagyl ER. The micromeritic properties, tablet evaluation properties, floating lag time and floating duration was found to be the best in F2. Moreover, drug release profile both for Ciprofloxacin Hcl and Metronidazole is almost similar to that of the target profiles and hence it can be very well concluded that F2 is the optimized batch. This would further conclude that the best suited ratio between the polymers HPMCK100M and HPMCE10M would be 2:1 and the levels of the polymers best suited for the ideal formulation would be 133.3mg and 66.6mg for HPMCK100M and HPMCE10M respectively. Guar gum concentration of 3.3 % which is equivalent to 50 mg was found to be the ideal concentration for the desired floating lag time and to achieve floating duration of more than 20 hours. The statistical parameters measured in the one-way ANOVA test is depicted in table IV. Based on the p-values, F-

value, R^2 value and the S value almost the entire study data has no statistically significant differences between group means. For the study of concentration of guar gum on floating lag time, the p value is less than 0.05 thus rejecting the null hypothesis and concludes that not the entire population means are equal. The surface plots of any two factors at a time with the given response are depicted in figures III, IV and V. After 3 month stability studies, the appearance of the optimized batch F2 was found to be within the specification and not affected by the stability conditions. Floating lag time was found to increase at the end of every month with values of 78 seconds, 82 seconds and 94 seconds at the end of 1, 2 and 3 months respectively. There was a gradual but slow decrease in the % drug release of both Ciprofloxacin Hcl and metronidazole after every month. This decrease was very less and fulfilled the objective of more than 90 % drug release after 20 hours of drug dissolution. The % drug release of Ciprofloxacin Hcl at the end of 20 hours was found to be 97.53 %, 97.04 % and 96.98 % at the end of 1, 2 and 3 month respectively as compared to % drug release of 98.93 % initially. The % drug release of metronidazole at the end of 20 hours was found to be 94.11 %, 93.34 % and 92.92 % at the end of 1, 2 and 3 months respectively as compared to % drug release of 94.87 % initially. The pictorial representation of drug release profile at various intervals for initial, 1, 2 and 3 months is represented in figure VI and VII for Ciprofloxacin Hcl and metronidazole release respectively. This decrease in % drug release is not very significant thus indicating good stability of F2 at accelerated conditions of 40 ^{0}C / 75% RH.

Table 2: Micromeritic properties of powder blends of batches F1 to F8.

Powder Blend	Angle of repose (°)	Loose Bulk Density (g/ml)	Tapped Bulk Density (g/ml)	Carr's Index (%)	Hausner's ratio
F1	28.37	0.679	0.729	16.27	1.25
F2	27.64	0.634	0.714	16.59	1.22
F3	27.29	0.654	0.753	19.51	1.27
F4	25.83	0.613	0.737	17.93	1.21
F5	26.71	0.668	0.768	17.44	1.23
F6	25.68	0.654	0.726	16.28	1.21
F7	27.59	0.689	0.741	17.21	1.18
F8	26.21	0.613	0.792	17.63	1.26

Table 3: Hardness, friability, weight variation, assay, floating lag time and floating time of batches F1 to F8.

Batch number	Hardness (Kg/cm^2) (n=10)	Friability (%) (n=10)	Weight variation(mg) (avg±%SD) (n=20)	Assay (90-110%) (n=3)	Floating lag Time (S)	Floating Time (h)
F1	4.5±0.19	0.39±0.58	99.7±1.57	97.6±1.8	62	>20
F2	4.7±0.53	0.41±0.91	100.1±0.89	97.1±2.7	75	>20
F3	4.8±0.81	0.48±0.53	99.8±1.15	101.7±0.9	69	>20
F4	4.8±0.46	0.51±0.15	99.8±1.07	98.2±1.3	60	>20
F5	5.0±0.27	0.45±0.47	100.2±2.34	99.3±1.1	79	>20
F6	4.6±0.89	0.51±0.12	100.1±2.5	97.1±2.3	81	>20
F7	4.7±0.29	0.45±0.19	99.9±1.2	98.7±1.4	83	>20

F8	4.8±0.31	0.43±0.21	99.8±1.6	101.5±2.8	69	>20

Table 4: Parameters of ANOVA test for all the factors and the responses

ANOVA	P value	F value	R^2	S
HPMCK100M on floating lag time	0.770	0.09	1.53	9.2
HPMCE10M on floating lag time	0.554	0.39	6.14	9.03
Guar gum on floating lag time	0.045	6.18	50.72	6.2
HPMCK100M on %drug release of Ciprofloxacin Hcl	0.852	0.04	0.63	1.1
HPMCE10M on %drug release of Ciprofloxacin Hcl	0.171	2.41	28.67	0.94
Guar gum on %drug release of Ciprofloxacin Hcl	0.512	0.49	7.48	1.07
HPMCK100M on %drug release of metronidazole	0.778	0.09	1.43	1.54
HPMCE10M on %drug release of metronidazole	0.623	0.27	4.29	1.5
Guar gum on %drug release of metronidazole	0.199	2.09	25.80	1.33

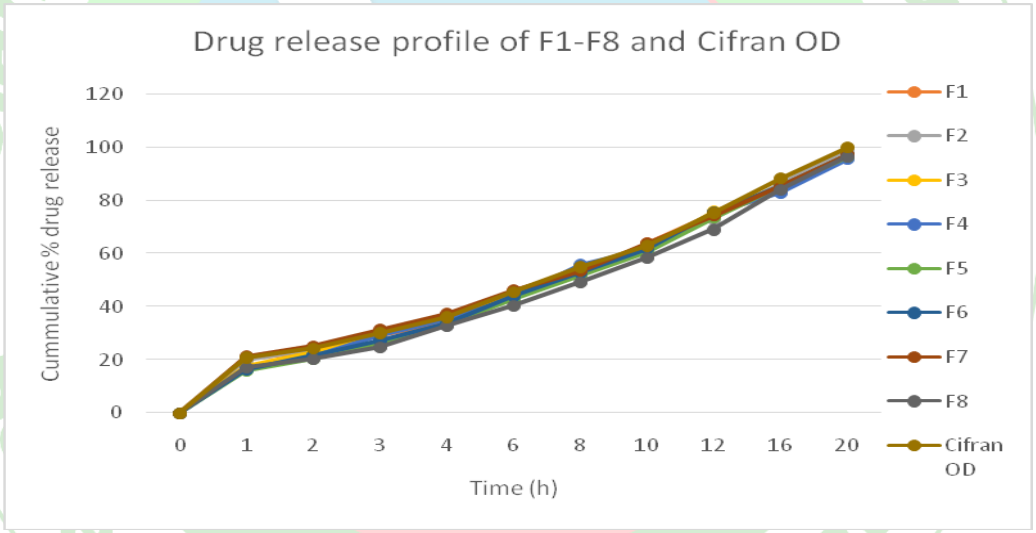

Figure 1: Drug release profile of Ciprofloxacin Hcl for batches F1-F8 and Cifran OD

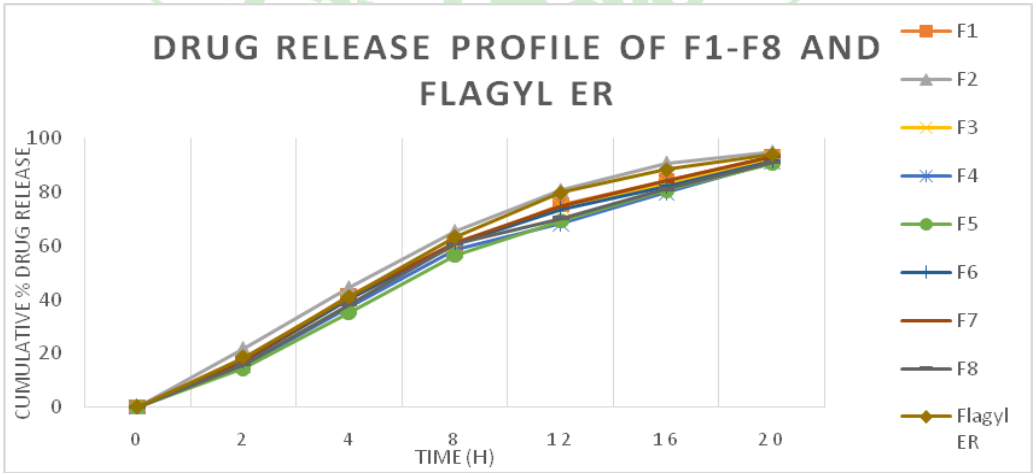

Figure 2: Drug release profile of Metronidazole for batches F1-F8 and Flagyl ER

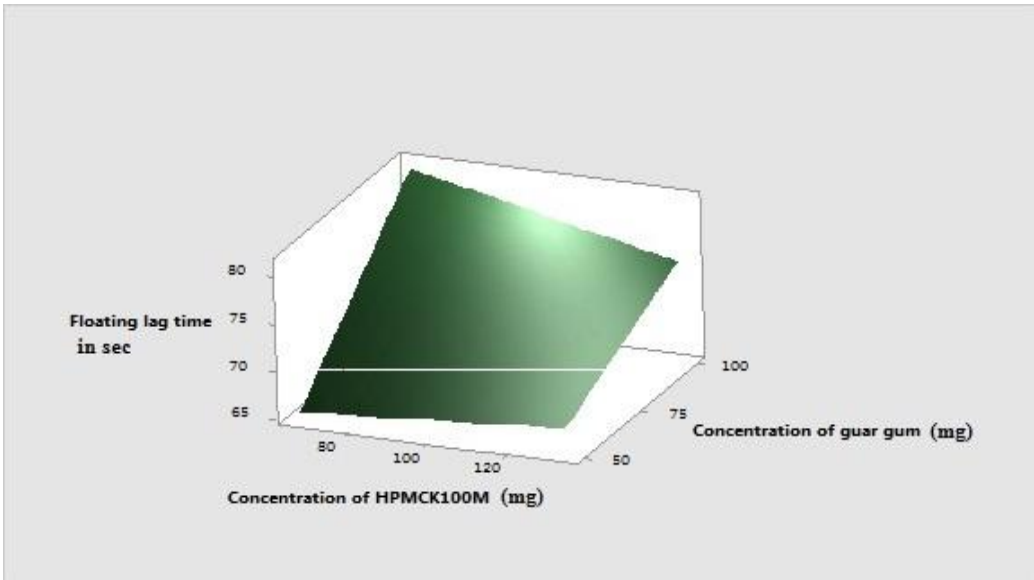

Figure 3: Surface plot showing the effect of concentration of guar gum and concentration of HPMCK100M on floating lag time in seconds.

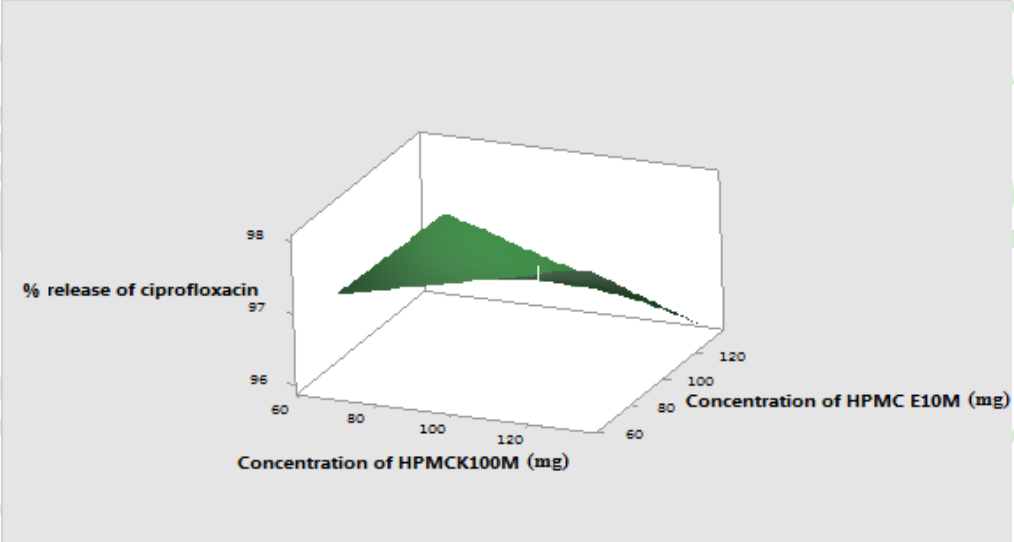

Figure 4: Surface plot showing the effect of concentration of HPMCE10M and concentration of HPMCK100M on % drug release of Ciprofloxacin Hcl

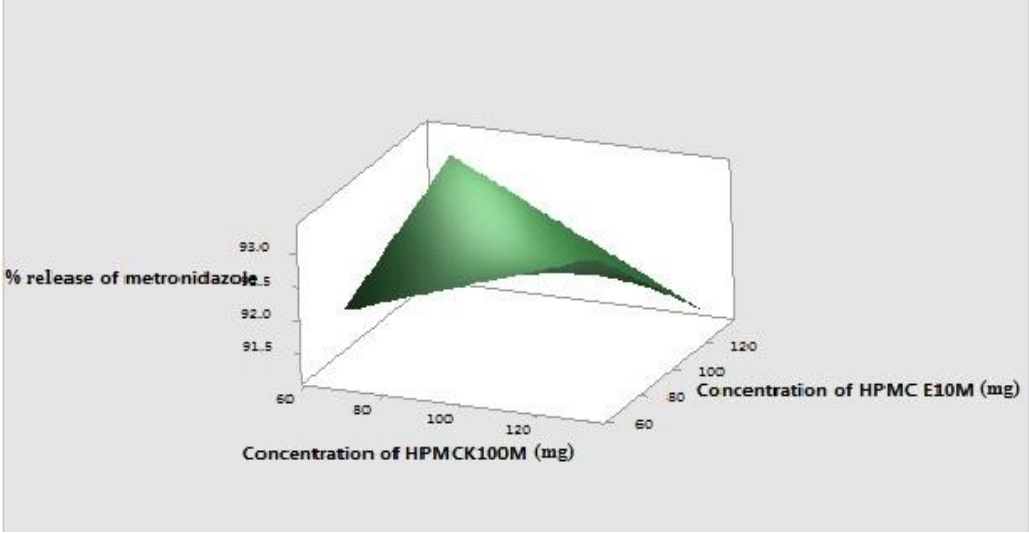

Figure 5: Surface plot showing the effect of concentration of HPMCE10M and concentration of HPMCK100M on % drug release of metronidazole.

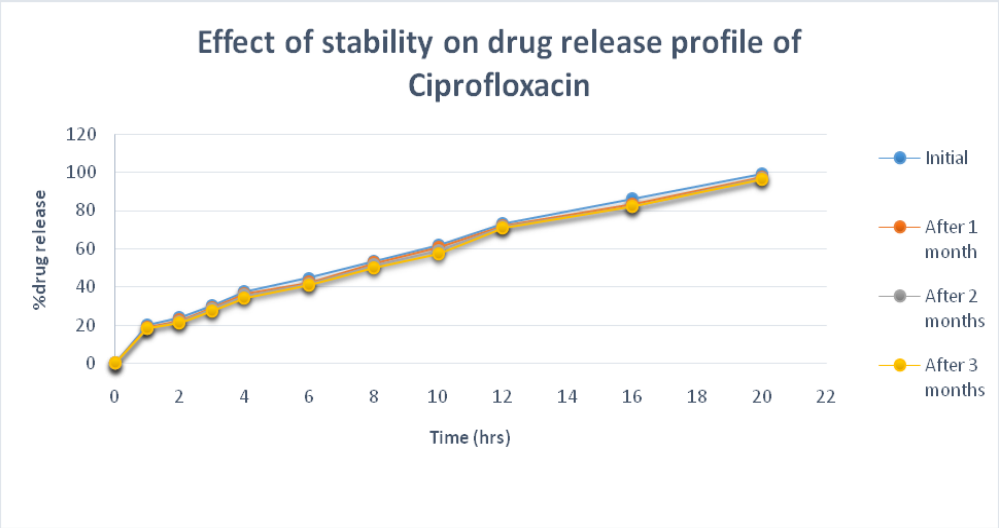

Figure 6: Plot of % drug release versus time in hours for F2 showing effect of stability studies on drug release profile of Ciprofloxacin Hcl

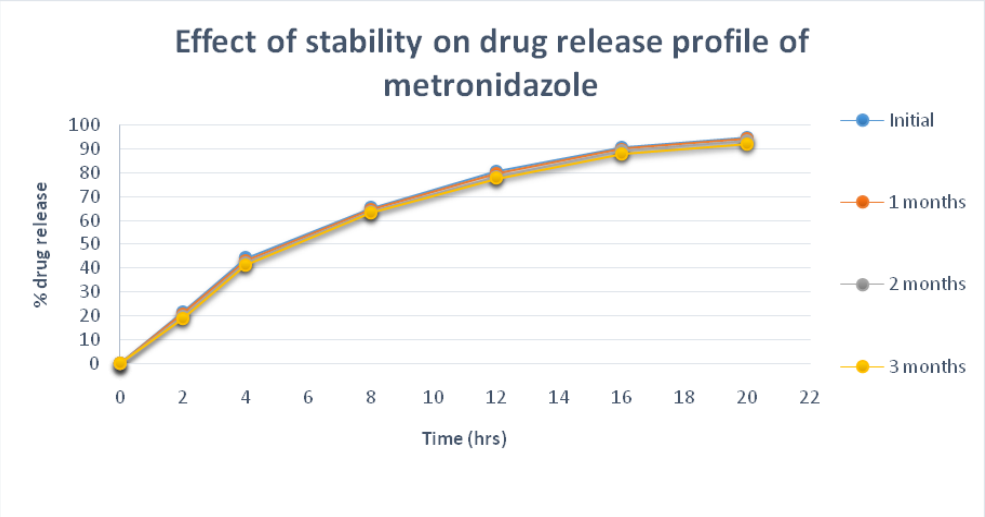

Figure 7: Plot of % drug release versus time in hours for F2 showing effect of stability studies on drug release profile of metronidazole

CONCLUSION

A combination of Ciprofloxacin HCL and metronidazole was thought to prove an efficient way of treating mixed aerobic/anaerobic infections also increasing capability of treating infections rather than individual administration. Development of gastro retentive dosage form can be advantageous, that can provide prolong gastric retention and increase efficacy of the dosage form. To optimize the formulation parameters, a 2^3 full factorial design with three factors and two levels of each factor was carried out to optimize the polymer ratio of HPMC K100M and HPMC E10M and to optimize the guar gum quantity. Most of the experiments carried out showed a linear and parallel release profile but batch F2 showed the desired floating lag time of 75 seconds along with a floating time of more than 20 hours. The drug release profile for both Ciprofloxacin Hcl and metronidazole also exhibited similarity to that of the marketed brands Cifran OD and Flagyl ER. Based on the floating lag time and drug release profiles it was clear that the ratio of polymers HPMCK100M and HPMC E10M in the ratio of 2:1 and the guar gum concentration of 3.3 % was the ideal combination to achieve

the desired floating lag time, floating duration and drug release profiles for Ciprofloxacin Hcl and Metronidazole. Formulation F2 value of F2 was nearing 100 which indicated a close resemblance with point to point dissolution matching of the test batch to the target profile. Based on the ANOVA test, the study between concentration of guar gum and floating lag time, the p value was found to be less than 0.05 thus rejecting the null hypothesis. For the other studies, there were no statistically significant differences between group means. The stability of the optimized batch revealed not much difference in the floating lag time and drug release profiles after 3 months thus indicating good stability at 40 ^{o}C / 75% RH.

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