https://doi.org/ 10.48047/AFJBS.6.5.2024. 9759-9790

African Journal of Biological Sciences

DESIGNING OF RESPONSE SURFACE AND CONTOUR PLOT BY APPLYING MATHEMATICAL AND STATISTICAL METHODS IN FORMULATION OF OFLOXACIN BASED GASTRORETENTIVE FLOATING DRUG DELIVERY SYSTEM

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Article History
Volume 6, Issue 5, 2024
Received: 15 May 2024

Accepted: 02 Jun 2024
doi: ***10.48047/AFJBS.6.5.2024. 9759-9801***

ABSTRACT:

Ofloxacin is a synthetic broad-spectrum antimicrobial agent for oral administration. Ofloxacin is considered to be soluble in aqueous solutions with pH between 2 to 5. It is sparingly to slightly soluble in aqueous solutions with pH 7 (solubility falls to 4 mg/ml). The molecule exists as a zwitterion and precipitation of active compound occurs in small intestine which adversely affects absorption. Conventional formulations of Ofloxacin cannot maintain required plasma concentration for long time and is therefore given 3-4 times a day also it shows greater dose dependent side effects. So need to formulate such drugs into desirable drug delivery systems. This study investigated utility of a 3-factor, 3-level Box-Behnken design and optimization process for floating tablet. Amount of HPMC, amount ofNaHCO3 and amount of citric acid were selected as the independent variables whereas total floating time (TFT), T50%, % CR10hrs, and diffusion coefficients (n) were selected as dependent variables. The prepared tablets of Ofloxacin were evaluated for dissolution study andfound to follow zero order release kinetic. The responses were analyzed using ANOVA and the individual response parameters were evaluated using F test and polynomial equation was generated for each response using multilinear regression analysis (MLRA). Theamount of HPMC and amount of citric acid were found tosignificantly influence all response parameters selected whereas the amount of NaHCO3 has significant effect on TFT. The resultant data were critically analyzed to locate the composition ofoptimum formulations. All predicted values of response variables of optimized formulation demonstrated close agreement with the experimental data during optimization procedure.

Keywords: GRFDDS, Box-Behnken Design, Ofloxacin, Optimization

INTRODUCTION:

The gastroretentive drug-delivery system can be retained in the stomach and assists in improving the oral sustained delivery of drugs that have an absorption window in a particular region of the GI tract. These systems help in continuously releasing the drug before it reaches the absorption window, thus ensuring optimal bioavailability and peak plasma concentration. Several approaches are currently used to prolong gastric retention time. These include floating drug-delivery systems, swelling and expanding systems, polymeric bioadhesive systems, high-density systems, and other delayed-gastric-emptying devices. From the formulation and technological point of view, the floating drug delivery system (FDDS) is considerably easy and logical approaches in development of gastroretentive dosage forms (**1**).

Bioavailability of Ofloxacin is strongly dependent on local physiology in G.I.T. and is preferably absorbed in the higher section of intestine. Ofloxacin is readily soluble in intestinal fluid, where neutral to slightly alkaline pH condition prevail; however, precipitation of active compound occurs, which adversely arrests absorption in the lower section of the intestine. There is a need for system that resides in the stomach over a relatively long time & release the active compound there in a sustained manner (**2**).

In the development of GRFDDS many trial and error methods are used, number of formulations has to be prepared to get a conclusion, which involves lot of money, time and energy. These can be minimized by the use of optimization technique. The optimization techniques are best to avert by correlating several experimental variables. Experimental design is a statistical design that prescribes or advises a set of combination of variables **(3).** Formulation design may be interpreted on the basis of the values of controllable independent variables, which give the most desired value of dependent variables. Depending on the number of factors, their levels, possible interactions and order of the model, various experimental models are chosen. Among them Box-Behnken model is easy to predict and provide all possible information for correlation of various variables. The model is validated using ANOVA calculation, and then the pure error measurement is done. The variance of these observations pooled over all to get an estimate of pure error of

variance. Once a model is selected and validated, the brute force method is applied for the prediction of response. With the help of 3D-response surface or a 2D contour diagram, the prediction is done using these graphs either by grid search or feasibility search methods **(4).**

The aim of research work is to design and evaluate controlled release floating tablet of Ofloxacin in view to enhance plasma concentration and therapeutic action. Mathematical optimization of the variable of formulation using response surface methodology and their evaluation to obtained reliable and reproducible product.

MATERIALS AND METHODS:

The active drug Ofloxacin obtained from Alkem pharmaceutical, vapi limited, Gujarat and other ingredients such as HPMC, $NaHC0_3$, Lactose, and Citric Acid obtained from S.D. Fine Chemicals Ltd., Mumbai.

Drug Excipients Interaction Study

Infra red spectrometry is a useful analytical technique utilized to check the chemical interaction between the drug and the other excipients used in the formulations. The samples were powdered and intimately mixed with dry powdered potassium bromide. The powdered mixture was taken in a diffuse reflectance sampler and the spectra recorded by scanning in the wavelength region of 2.5 to 25 μ in a FTIR spectrophotometer (Jasco 460 plus, Japan). The IR spectrum of drug was compare with that of the physical mixture to check for any possible drug-excipients interaction **(5).**

Formulation Design

Box-Behnken designs are response surface designs, specially made to require only 3 levels, coded as -1, 0, and +1. Box-Behnken designs are available for 3 to 10 factors. This procedure creates designs with desirable statistical properties but, most importantly, with only a fraction of the experimental trials required for a three-level factorial. Because there are only three levels, the quadratic model was found to be appropriate **(6).**

Formulation of Floating Tablets

Ofloxacin, HPMC, $NaHCO_3$, citric acid and lactose were taken and passed through 44 mesh separately. The drug with other ingredients was dry mixed for the period of 10 minutes in a mortar to get uniform mixture powder. The mixture was blended with magnesium stearate for 2-3min to improve flow property. The powder was compressed into tablet weighing 500mg using 12.5 mm flat punches in a rotary tablet press to a hardness of 4.5 kg/cm^2**, (7).** The prepared tablets were found an average thickness of 3.5 _ 0.15 mm.

Floating Property Study:

The time taken for tablet to emerge on surface of medium is called the buoyancy lag time (BLT) and duration of time the dosage form constantly remain on surface of medium is called the total floating time (TFT).

One tablet from each batch was taken in USP XXIII type II dissolution apparatus containing 900 ml of 0.1 N HCl. The study was performed at the paddle rotational speed of 50 rpm and bath temperature of 37 ± 0.5 °C. The time taken for tablet to emerge on surface of medium and the duration of time the tablet constantly remain on surface of medium was recorded as the BLT & TFT respectively **(8).**

Invitro Drug release Study:

Dissolution of the tablet of each batch was carried out using USP XXIII dissolution type II apparatus using paddles at 50 rpm. 750 ml of pH 1.2 buffered dissolution medium was filled in a dissolution vessel and the temperature of the medium was set at 37 ± 0.5 ^{0}C. 5 ml of sample was withdrawn at predetermined time intervals and same volume of fresh medium was replaced. The withdrawn samples were diluted to 25 ml in volumetric flask containing 1 ml of ferric chloride reagent and analyzed by an UV spectrophotometer at 410 nm using a reagent blank prepared in similar manner taking 5 ml of water in place of sample solution **(9), (10).**

Scanning electron microscopy (SEM) of the optimized formulation:

The SEM analysis was conducted using Jeol, Japan (Model - JSM 5610LV) scanning electron microscope for the optimized formulation in the following states,

- Dry tablet surface and
- Tablets after swelling of 3, 6 and 9hrs.

As with SEM high vacuum is required for image formation and samples must be thoroughly desiccated before entering the vacuum chamber, therefore samples were thoroughly dried after swelling for analysis. The dried samples were mounted on sample holder using double sided adhesive carbon tape. The SEM was operated at 15 KV. The condenser lens position was maintained at a constant level. **(11), (12).**

Data analysis:

The response surface methodology is a collection of mathematical and statistical techniques used for modeling and analysis of problems in which a response of interest is influenced by several variable and the objectives is to optimize this response.

The run or formulation, which are designed based on Box-Behnken design are evaluated for the response. The response values are

subjected to multiple regression analysis to find out the relationship between the factor used and the response value obtained. The response values subjected for this analysis are:

1. Total floating time
2. $T_{50\%}$
3. % $CR_{10\ hrs}$
4. Diffusion coefficient (n)

The Diffusion coefficient (n) obtained after fitting the release rate to Korsmeyer and Peppas model. The multiple regression analysis was done using DESIGN EXPERT 6.0.11 (STAT-EASE) demo version software, which specially meant for this optimization process. Analysis of data was carried out using ANOVA and the individual parameter was evaluated with F-test. Using the regression coefficient of factor, the polynomial equation for the each response is generated **(13), (14).**

Optimization:

The computation for optimized formulation was carried using software, DESIGN EXPERT 6.0.11 (STAT-EASE). The response variable considered for optimization were total floating time, $T_{50\%}$, $\%CR_{10\ hrs}$, diffusion coefficient (n).The optimized formulation was obtained by applying constraints (goals) on dependent (response) and independent variables (factors) **(15), (16).** The optimized formulation is prepared and evaluated for total floating time, $T_{50\%}$, $\%CR_{10\ hrs}$, diffusion coefficient (n). Observe response value of the optimized formulation is compared with predicted value.

Stability studies of optimized formulation:

ICH specified the length of study and storage conditions. The optimized formulation was packed in amber-colored bottle, which was tightly plugged with cotton and capped. It was then stored at 40° C / 75 % RH for 6 months. The formulation was evaluated for hardness, drug content, floating properties, dissolution study and compare with original formulation **(17).**

RESULTS AND DISCUSSION:

Floating drug delivery system belongs to oral controlled drug delivery system group that are capable of floating in the stomach by bypassing the gastric transit. These dosage forms are also defined as Gastro Retentive drug delivery system or hydrodynamically balanced dosage form or gastric floating drug delivery system, which can float in the contents of the stomach and release the drug in a controlled manner for prolonged period. The release rate will be controlled depending upon the type and

concentration of the polymer that swells, leads to diffusion and erosion of the drug **(18).**

Ofloxacin is a synthetic broad-spectrum antimicrobial agent for oral administration. Ofloxacin is considered to be soluble in aqueous solutions with pH between 2 to 5, it is sparingly to slightly soluble in aqueous solutions with pH 7 (solubility falls to 4 mg/ml). At the pH conditions in the small intestine the molecule exists as a zwitterion, also precipitation of active compound occurs, which adversely arrests absorption in the lower section of the intestine. In view of this absorption characteristic, the hypothesis of current investigation is that if the gastric residence time of Ofloxacin containing formulation is prolonged and allowed to float in the stomach for a long period, the oral bioavailability might be increased **(19).**

The polymers were selected in such a way that one will give initial burst release, which is essential for achieving therapeutic level, while the other will control the drug release by maintaining buoyancy. The conventional tablet formulations release the drug slowly in the stomach for gradual absorption in the intestines. The slow but complete drug release in the stomach of the drugs which are having the absorption window in the stomach is definitely expected to increase bioavailability of the drug as well its complete utilization which may results to lower the dose, frequency and GI side effect **(20).**

GRFDDS formulation of Ofloxacin would be of great use and therefore Ofloxacin floating delivery was prepared by incorporating the drug and polymer in one layer, and the gas generating agent and polymer in another layer, then compressing both into a single unit formulations were optimized by using software package. On the basis of preliminary identification test it was concluded that the drug complied the preliminary identification. There was a no drug excipients interaction, which was conformed by the IR spectra of drug and physical mixture. **(Fig.no-1 a, b).**

The viscosity of HPMC (2% w/v) in water was found to be 4100 cps, which would sufficient to maintain the integrity of the matrix **(20), (21).** The free flowing nature of drug and excipients was clearly evident from the different flow properties. A 3-factor 3-level Box-Behnken design was used for the formulation of tablets. The independent factors used in the design are amount of HPMC, $NaHCo_3$ and Citric acid **(Table 1).** The Box-Behnken design **(Table 2)** is formed by combining two-level factorial designs with incomplete block designs. The

optimized formulation was obtained by applying constraints (goals) on dependent (response) and independent variables (factors). The selected limit values (Upper, middle and lower) were used to optimize the formulations coded in (**Table 3).** The results of buoyancy lag time and total floting time **(Table 4)**. All the batches of tablet formulations (except batch F2) were found to exhibit short buoyancy lag time (maximum buoyancy lag time recorded were 86 ± 7 second). The short buoyancy lag time can be due to presence of sodium bicarbonate and citric acid. Sodium bicarbonate and citric acid were used in combination to minimize the lag time in fabrication of GRFDDs the tablet of batch F2 exhibited a longer buoyancy lag time of 20.78 minutes. This can be due to the presence of $NaHCO_3$ at low level and HPMC at high level. The high level of HPMC would possibly prevents the entry of media into the tab matrix and prolong the buoyancy lag time. The effect of concentration of HPMC on floating has been reported in literature **(4).**

The results of total floating time (TFT) of all batches of tablet were found to exhibit maximum floating time i.e. 10 hours. Tablets of batch F1, F3, F7 and F12 exhibited short floating time i.e. 3-5 hours because they eroded faster in media due to high amount of $NaHCO_3$ and Citric acid in coupled with less amount of HPMC. ANOVA **(Table 5).**The model F-value of 17.56 implies that model is significant. There is only 0.05% chance that a model F-value this could occur due to noise. Polynomial equation in term of coded factors.

TFT= 10 + 1.91A – 0.66B – 1.25C – 1.39A^2 – 0.97B^2 – 0.07C^2 + 0.27AB + 1.46AC – 1.04BC --- (1) R^2 = 0.9576

For the TFT of the formulations demonstrates that Value of "Prob > F" less than 0.05 indicate factor A, B, C, AC, BC had significant effect on total floating time.

The amount of HPMC increased, TFT increased due to increased matrix integrity at high amt of HPMC while amt of $NaHCO_3$ and citric acid increases, TFT decrease because $NaHCO_3$ and citric acid promote faster erosion of tablets. So the presence of optimum amount of HPMC, $NaHCO_3$, and citric acid is important in achieving good floating time and minimum buoyancy lag time. The physical parameters of tablets showed that the tablets of all batches had desirable physical characteristics.

The relationship between the dependent and independent variables was further elucidated using one factor plots **(Fig. 2a,b,c)** ,contour

plots and response surface plots **(Fig. 3a,b)** shows that at a fixed level of $NaHCO_3$ (50 mg), TFT decrease from 10 to 5.14 hrs at low level of A (amount of HPMC) and high level of C (citric acid). However at high level of A (amount of HPMC) TFT remains unaffected with change in amount of citric acid. These might be due to at low level of HPMC (75mg), matrix unable to remain intact with increase in citric acid. The interaction effect of B (amount of $NaHCO_3$) and C (amount of citric acid) at a fixed levels of A (100 mg) **(Fig 4a,b).**The TFT decrease at high levels of B and C whereas at low levels, TFT remains high unaffected to change each other.

Time required for 50 % drug to get released ($T_{50\%}$) and $\%CR_{10hrs}$ were found to be in range of 0.7 to 8.6 hours and 57.35 ± 3.89 to 99.93 ± 0.07 respectively.

The model F-value of 14.04 implies that model is significant. There is only 0.11% chance that a model F-value this could occur due to noise. Polynomial equation in term of coded factors.

$$\mathbf{T_{50\%} = 7.3 + 2.48A - 0.71B - 1.66C - 1.84A^2 - 1.56B^2 - 0.44C^2 + 0.35AB + 1.5AC - 2.42BC \text{ ---- (2) } R^2 = 0.9475}$$

Value of "Prob > F" less than 0.05 indicate factor A, C, AC, BC had significant effect on $T_{50\%}$ and $\%CR_{10hrs}$. (**Table 6 & 7**). One factor plots are shown in Fig. **(Fig 5 a, b, c, d).** It was shown that as the amount of polymer increased, $T_{50\%}$ of formulations increased, whereas $\%CR_{10hrs}$ decrease.

The model F-value of 22.70 implies that model is significant. There is only 0.05% chance that a model F-value this could occur due to noise. Polynominal equation in term of coded factors

$$\mathbf{\%CR_{10\ hrs} = 69.06 - 13.68A + 2.64B + 9.5C + 7.55A^2 + 7.45B^2 - 3.47C^2 - 0.54AB - 6.32AC + 10.92BC \text{ ------ (3) } R^2 = 0.9669}$$

It was noticed that the matrix became more intact which slowed down the water uptake resulting in poor water diffusion and poor drug release. As the amount of citric acid increased, it reacted with $NaHCO_3$ producing effervescence and rendering the matrix more porous. This resulted in an increased $\%CR_{10hrs}$ and decrease $T_{50\%}$ from the porous tablet matrix.

Response surface plots and Contour plot **(Fig 6 a, b, c, d).** indicated that at a fixed level of B(50 mg) and low level of A (amount of HPMC), % CR_{10hrs} increases from 68.11 to 90.00 % and $T_{50\%}$ decrease from 6.86 to 1.66 as the amount of citric acid (C) increases from 0 to 10 mg. However simultaneous increasing amount of HPMC and amount of citric acid had no significant effect on % CR_{10hrs} and T 50%.

The interaction effect **(Fig 7 a, b, c, d).** of B (amount of $NaHCO_3$) and C (amount of citric acid) at a fixed levels of A (100mg) indicated that % CR_{10hrs} increases whereas T 50% decrease at high levels of both B and C. this can be attributed to formation of compact matrix with increasing level of HPMC and porous matrix with increasing level of $NaHCO_3$ and citric acid.

The dissolution data treatment of different batches of tablet (**Table 8**). The dissolution data of most of formulation fitted well into zero order release kinetics. The data fitment (**Table 9**) of the dissolution profiles done according to korsmeyer peppas model indicating the values of diffusion coefficients obtained range from 0.06 to 1.55. The formulation F1, F3, F7 and F12 which exhibited an initial burst phase showed a low value of diffusion coefficients ranging from 0.06 to 0.32. Low level of HPMC coupled with high amount of $NaHCO_3$ and citric acids for these formulations were responsible for the incompatibility of the system to control the release of Ofloxacin from the GRFDDS. Other tablet formulation gave relatively higher n value for diffusion coefficient ranging from 0.75 to 1.55. The mechanism of drug release in these cases was known to follow case II transport mechanism i.e. characterized by both erosion and diffusion **(22).**

The ANOVA **(Table 10)** for the diffusion coefficient (n) of the formulations demonstrates that Values of "Prob > F" were less than 0.05 indicating the factor A, C, AC, BC had significant effect on diffusion coefficient (n).

The Model F-value of 18.91 implies the model is significant. There is only a 0.04% chance that a "Model F-Value" this large could occur due to noise. Polynomial equation in term of coded factors

$$\mathbf{R^2 = 0.89 + 0.36A + 0.026B - 0.33C - 0.075A^2 - 0.16B^2 + 0.14C^2 + 0.15AB + 0.33AC - 0.29BC} \text{ -------- (4) } \mathbf{R^2 = 0.9889}$$

One factor plot **(Fig. 8)** shows that as HPMC level increased, the drug delivery system gained more control over the release of Ofloxacin, resulting in an increased diffusion exponent value. Citric acid was found to exert an opposite effect on the diffusion coefficient, which is clearly evident from the negative value for the regression coefficient in polynomial equation. An increased amount of citric acid could cause a decrease in value of diffusion exponent (n) by initiating the formation of porous matrix tablet. An optimum amount of citric acid in delivery device could be maintained without compromising drug

release by precisely monitoring the levels of $NaHCO_3$ and HPMC.

However simultaneous increasing amount of HPMC and amount of citric acid had no significant effect on diffusion coefficient (n). This can be attributed to opposite effect of amt of HPMC and citric acid. The interaction effect **(Fig. 9 & 10)** of B (amount of $NaHCO_3$) and C (amount of citric acid) at a fixed level of A (70mg) indicated that diffusion coefficient (n) increases at low levels of both B and C. These can be attributed to formation of compact matrix with increasing level of HPMC and porous matrix with increasing level of $NaHCO_3$ and citric acid.

For the optimization of floating tablets of Ofloxacin in constraints was fixed for all factors and response **(Table 11).** Constraints were set according to formulation of floating tablets using minimum amt of excipients, which will give desired response values. In the present study our aim was zero order drug release from the tablets and so that the diffusion coefficient was targeted to 1.

The optimized formulation was prepared after applying above criteria and observed response values was compared with predicted values. Comparison chart of observed and predicted values **(Table 12).** The predicted values of TFT had indicated that tablet would erode in 8.8 hours. But during dissolution study it was observed that a very small tablet was there at end of study and this will lead to high % error. However other response exhibits negligible values of % Error.

The dissolution data of optimized formulation fitted well into zero order release kinetics (r^2 = 0.9942) and korsmeyer peppas model (r^2 = 0.9992). The regression values and diffusion coefficients (n) values 0.91 i.e. nearest to 1 indicated that floating tablets follow zero order kinetics of drug release. The mechanism of drug release in these cases was known to follow case II transport mechanism i.e. characterized by both erosion and diffusion **(22).**

Stability study was performed for optimized formulation and it was found that formulation was stable for 6 months at 40 °C/ 75% RH. The formulation was found to be stable in terms of morphology, drug content and drug release.

The dissolution data of tablet formulations including optimized formulation (OF) and stability batch optimized formulation (OFS) **(Table 8)** and % CR vs. time plot for formulations **F1-F17 (Fig 11)**, % CR vs. time plot of **OF** and **OFS (Fig 12).** It was clear from dissolution profiles that the tablets of batch F3, F7, and F12 exhibits

initial burst phase during the first hour of dissolution. The burst phase was followed by a limited drug release for the rest of the period. The initial burst release can be attributed to low levels of HPMC combined with high levels of $NaHCO_3$ and citric acid. It was observed during the dissolution studies that tablets of all three batches eroded quickly with increased effervescence. Similar kind of quick erosion of tablet matrix was observed with high level of $NaHCO_3$ and citric acid in the formulation of floating tablet of calcium carbonate **(23),** other formulation showed a linear pattern of drug release from floating tablet.

The dissolution release kinetic study of all formulations [including stability batch (OFS)]

(Table 9). The dissolution data of most of formulation fitted well into zero order release kinetics. The data shows dissolution profiles fits with korsmeyer peppas model indicating the values of diffusion coefficients obtained range from 0.06 to 1.55. The formulation F1, F3, F7 and F12 which exhibited an initial burst phase showed a low value of diffusion coefficients ranging from 0.06 to 0.32. Low level of HPMC coupled with high amount of $NaHCO_3$ and citric acids for these formulations were responsible for the incompatibility of the system to control the release of drug from the GRFDDS. Other tablet formulation gave relatively higher n value for diffusion coefficient ranging from 0.75 to 1.55. The mechanism of drug release in these cases was known to follow case II transport mechanism i.e. characterized by both erosion and diffusion **(24).** The optimized formulation was prepared after applying all above criteria that considered in formulation of different batches. The dissolution data of optimized formulation fitted well into zero order release kinetics (r^2 = 0.9942) and korsmeyer peppas model (r^2 = 0.9992). The regression values and diffusion coefficients (n) values 0.93 i.e. nearest to 1 indicated that floating tablets follow zero order kinetics of drug release. The mechanism of drug release in these cases was known to follow case II transport mechanism i.e. characterized by both erosion and diffusion. Scanning electron microscopy of the formulation was mainly carried out for examination of surface of polymeric drug delivery system which provide important information about the porosity and microstructure of the device. From the scanning it was observed that as the time increases the swelling and the porosity of the tablet was increased which

was mainly helps to drug release **(Fig. 13 a, b, c, d).**

In respect to all above study the summeriesd data of variation in **TFT, T 50%**, **%CR $_{10\ hr}$** and **Diffusion coefficient (n)** due to change in concentration (amt of HPMC, $NaHCo_3$ and citric acid) of polymer **(25). (Table 13).**

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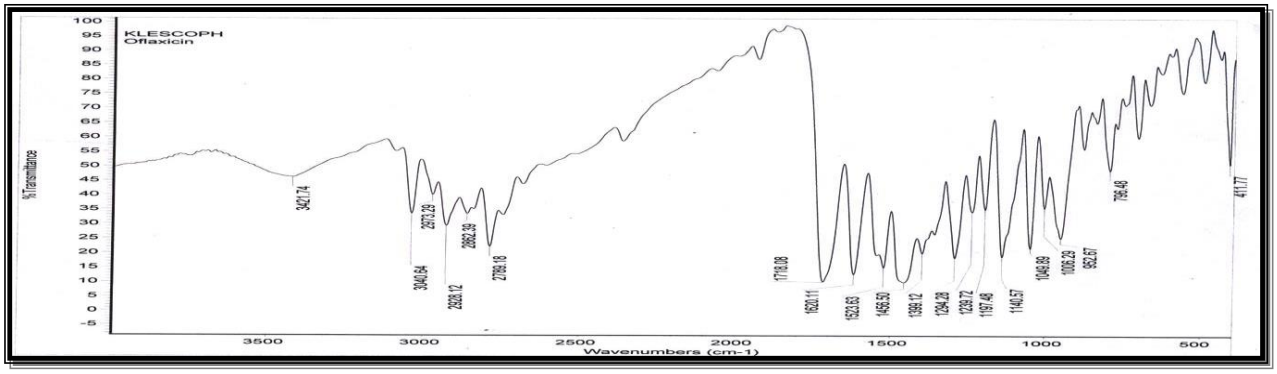


Fig 1a. FTIR Spectra of Ofloxacin

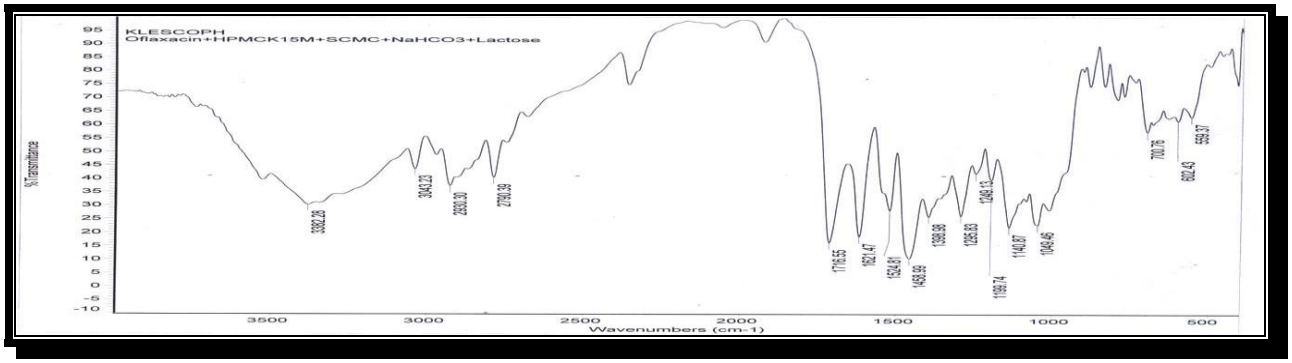
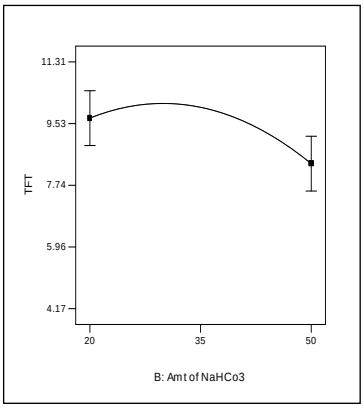
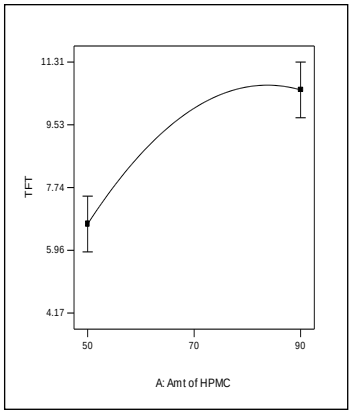


Fig 1b. FTIR Spectra of Ofloxacin + Polymer+ Excipients





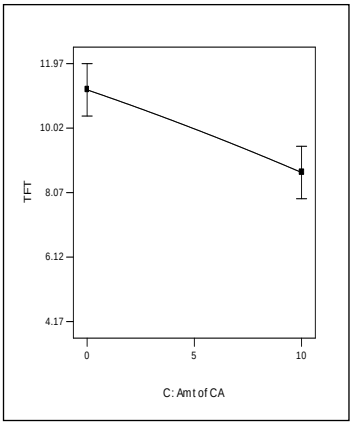
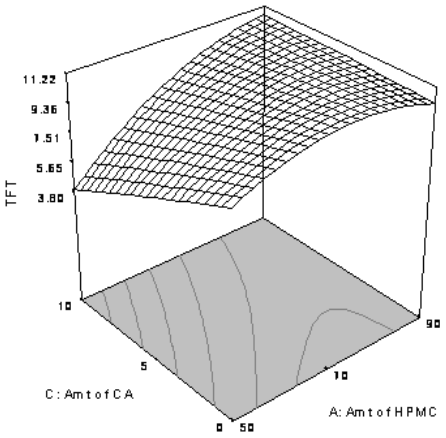


Fig **2 (a)** Fig **2 (b)** Fig **2(c)**

Fig 2. One Factor plot showing the effect of (a) HPMC (b) $NaHCo_3$ (c) Citric acid on Floating time.



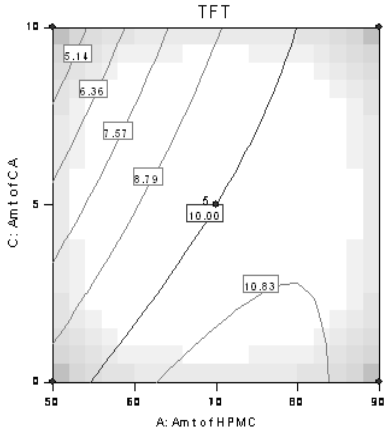
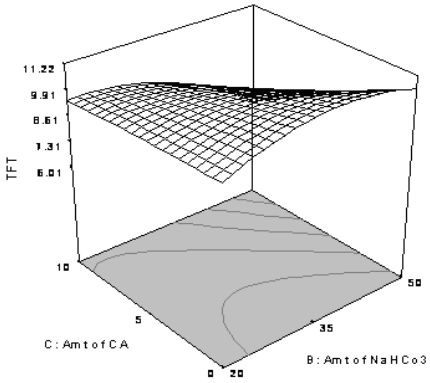


Fig **3 (a)** Fig **3 (b)**

Fig 3. (a) Response surface plot (3D) and (b) Contour plot showing the effect of the amount of HPMC and amount of citric acid on the total floating time.



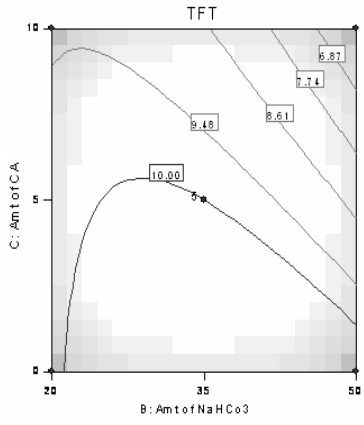
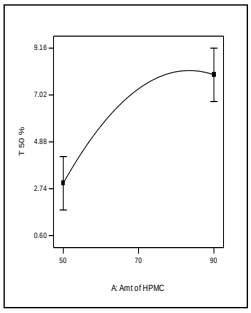
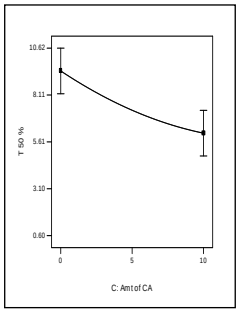
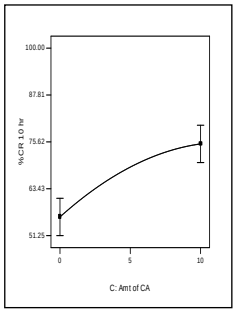


Fig **4 (a)** Fig **4 (b)**

Fig 4. (a) Response surface plot (3D) and (b) Contour plot showing the effect of the amount of $NaHCO_3$ and amount of citric acid on the total floating time.







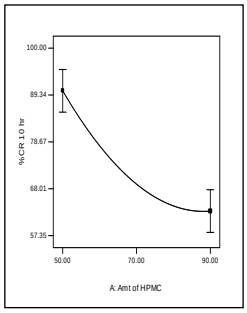


Fig **5 (a)** Fig **5(b)** Fig **5 (c)** Fig **5 (d)**

Fig 5. One Factor plot showing the effect of (a) HPMC (b) Citric acid on $T_{50\%}$ and % $CR_{10hrs.}$

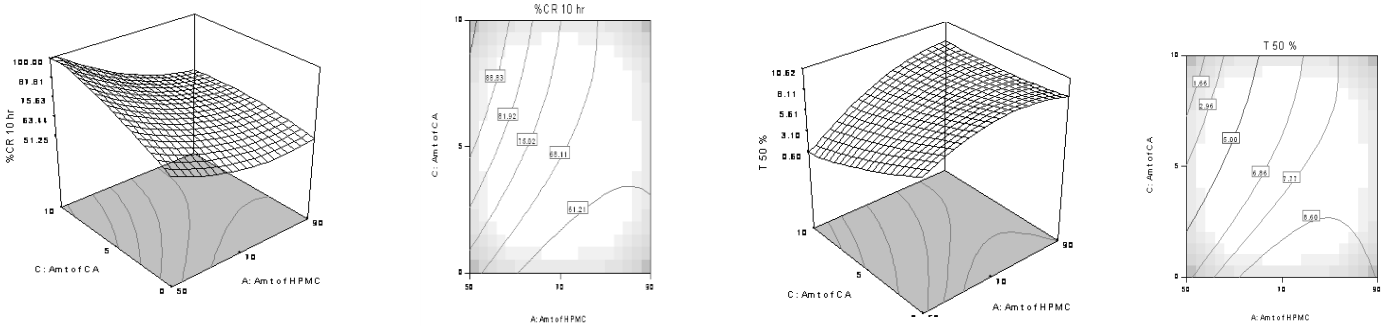


Fig **6 (a)** Fig **6 (b)** Fig **6 (c)** Fig **6(d)**

Fig 6. (a) Response surface plot (3D) and (b) Contour plot showing the effect of the amount of HPMC and amount of citric acid on the $T_{50\%}$ and $\%CR_{10hrs}$

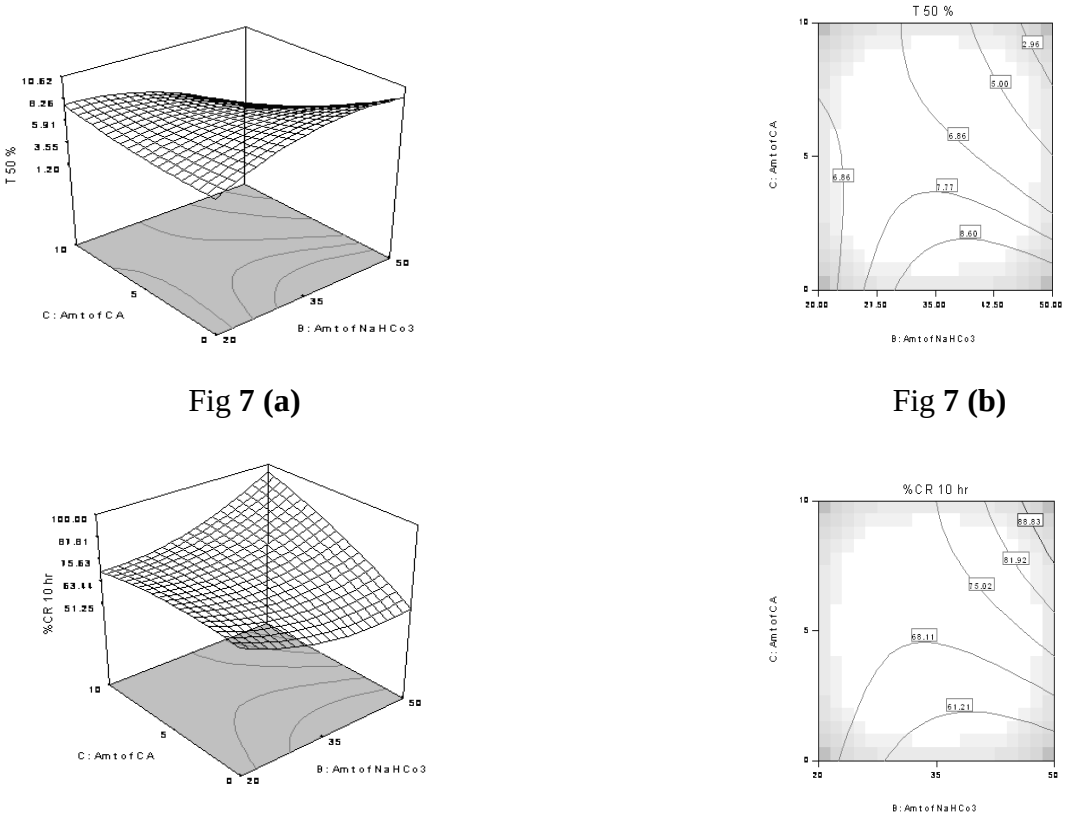


Fig **7 (a)** Fig **7 (b)**

Fig **7(c)** Fig **7 (d)**

Fig. 7. (a) Response surface plot (3D) and (b) Contour plot showing the effect of the amount of $NaHCO_3$ and amount of citric acid on the $T_{50\%}$ and $\%CR_{10hrs}$

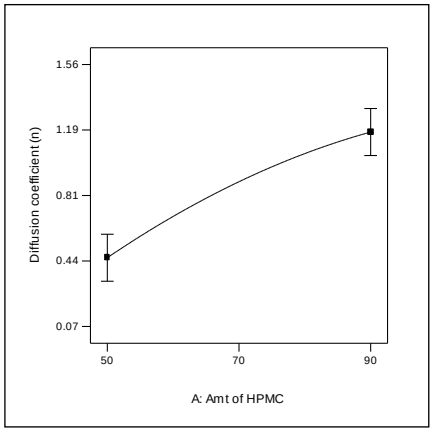


Fig **8 (a)**

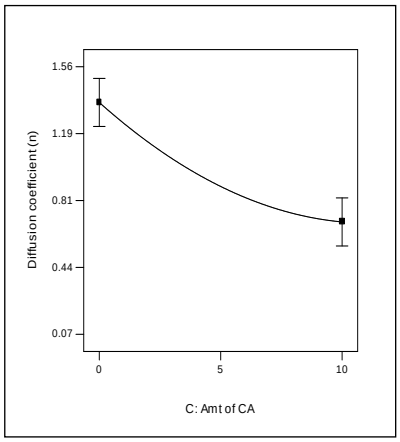


Fig **8 (b)**

Fig 8. One Factor plot showing the effect of (a) HPMC and (b) Citric acid on Diffusion coefficient (n).

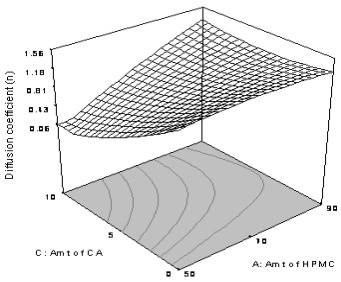


Fig **9 (a)**

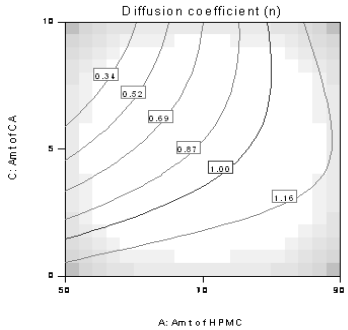


Fig **9 (b)**

Fig 9. (a) Response surface plot (3D) and (b) Contour plot showing the effect of the amount of HPMC and amount of citric acid on the Diffusion coefficient (n).

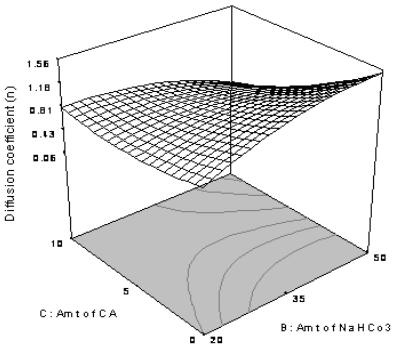


Fig **10 (a)**

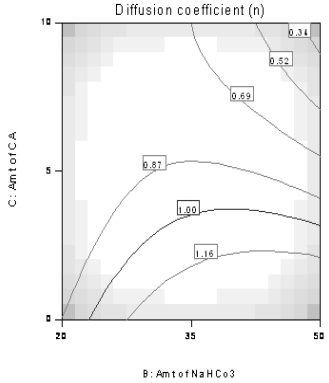


Fig **10 (b)**

Fig 10. (a) Response surface plot (3D) and (b) Contour plot showing the effect of the amount of $NaHCO_3$ and amount of citric acid on the Diffusion coefficient (n).

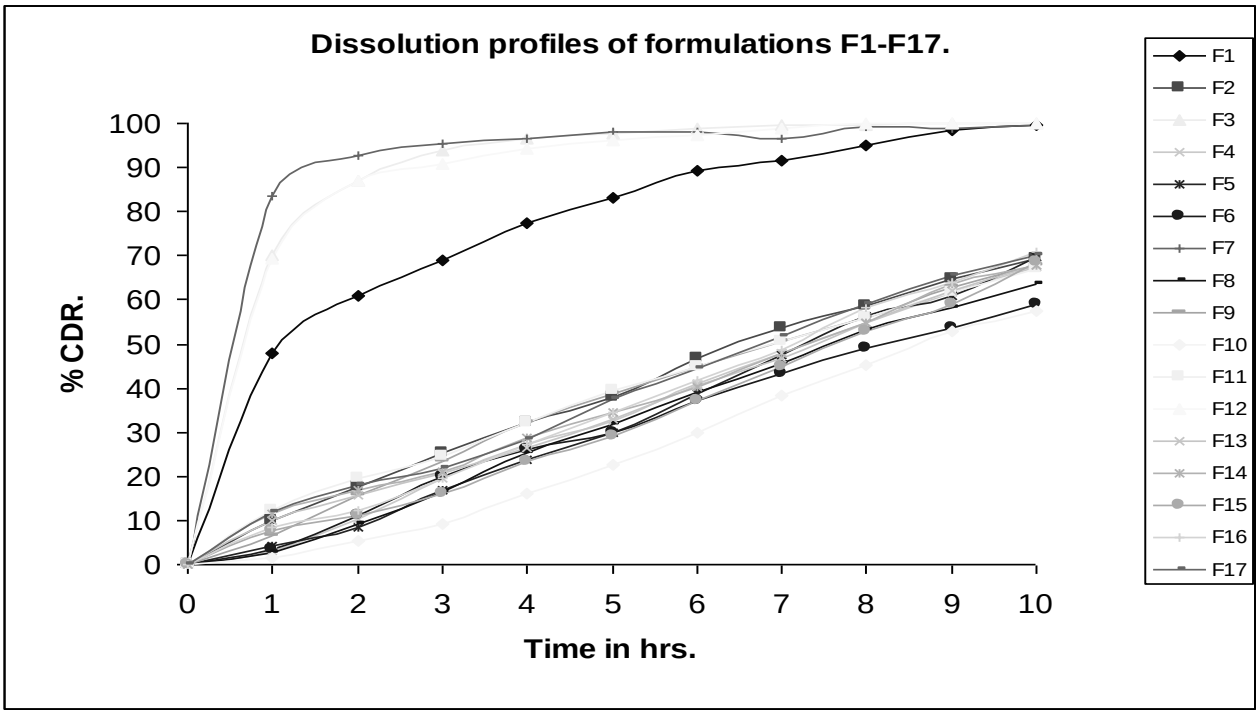


Fig 11. Dissolution profile of formulations F1-F17.

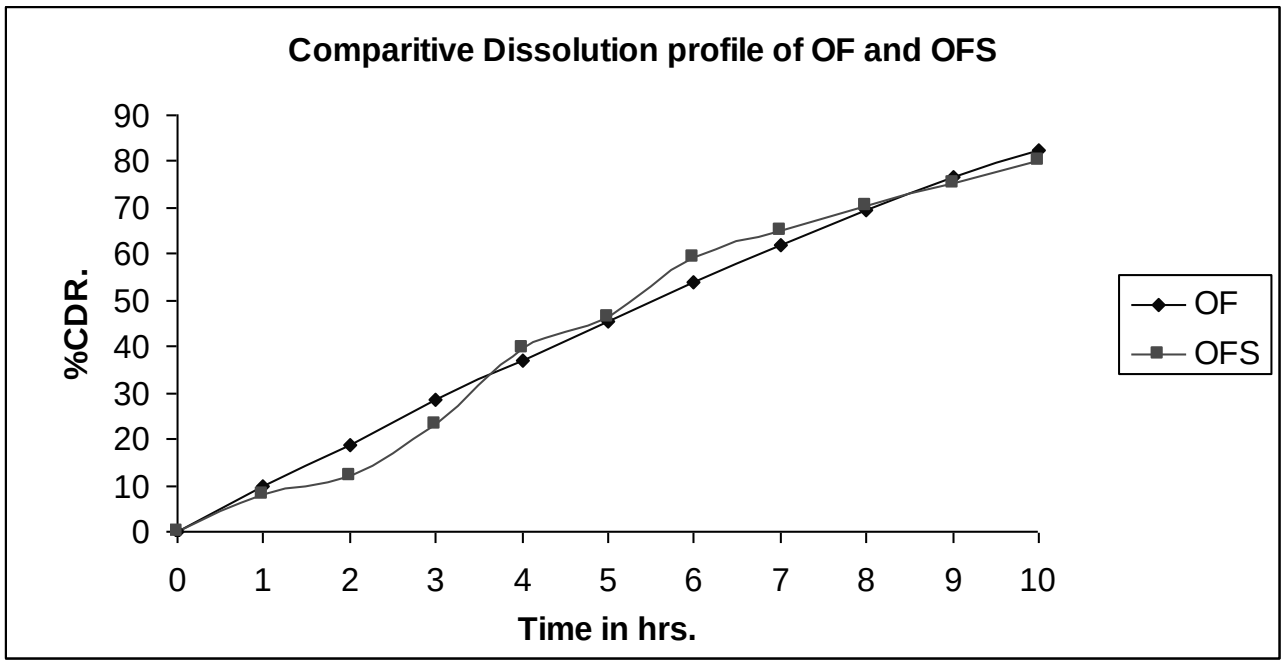


Fig 12. Comparative Dissolution profile of OF and OFS.

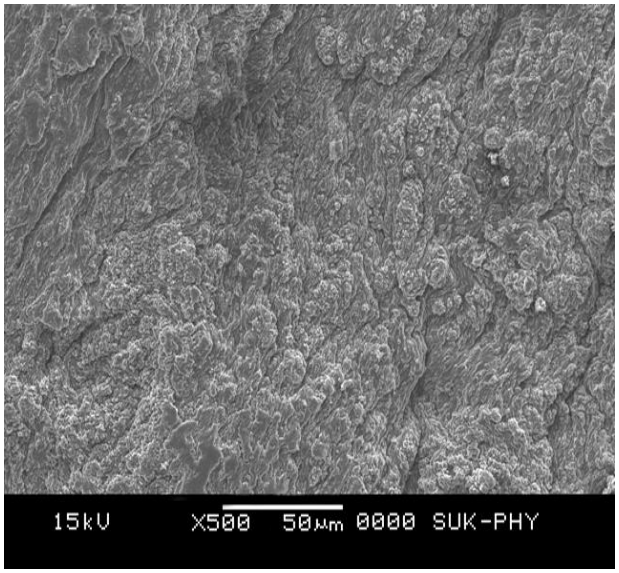


Fig **13 (a): dry surface**

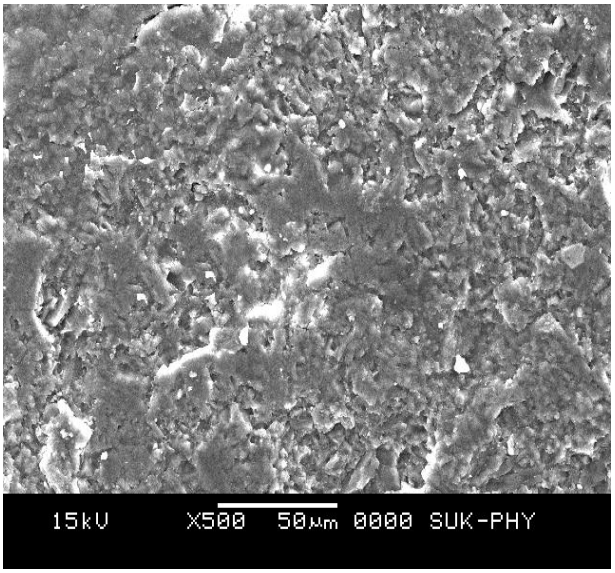


Fig **13 (b): after 3 hrs**

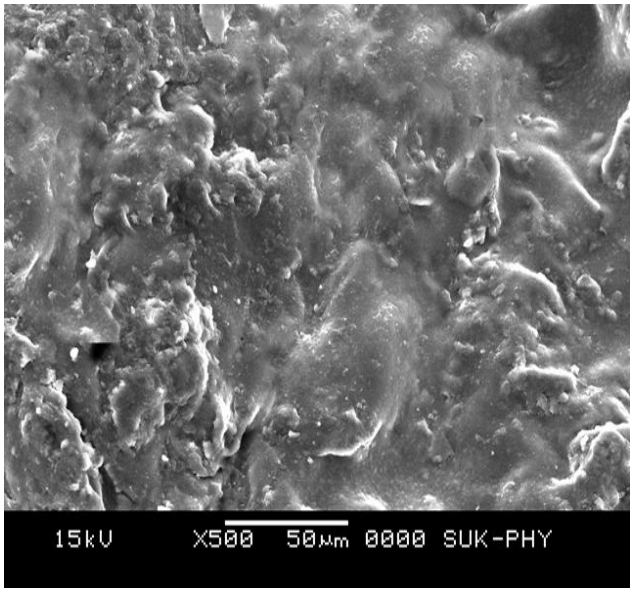


Fig **13 (c): after 6 hrs**

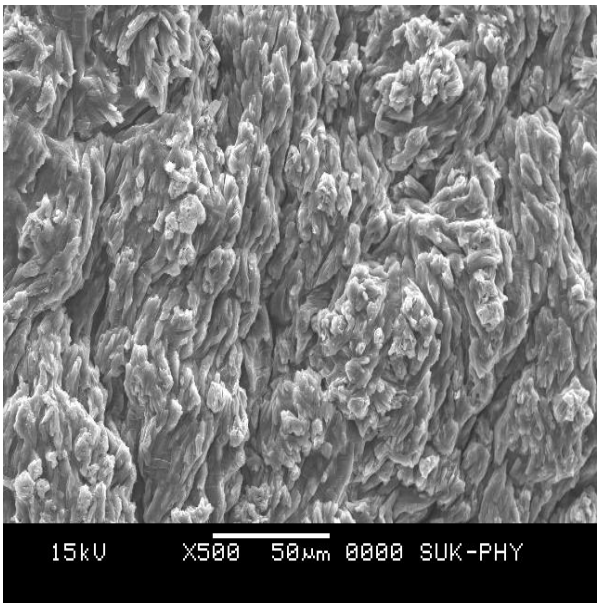


Fig **13 (d): after 9 hrs**

Fig 13. SEM of optimized formulation (OF) at various time intervals

Table I. Independent factors

Independent variable	*Levels*		
	Low	Middle	High
A = Amount of HPMC	75	100	125
B = Amount of $NaHCo_3$	30	50	70
C = Amount of Citric Acid	0	5	10

Table II. Box Behnken Design

Run	Coded value			Actual value		
	A	B	C	A	B	C
1	-1	-1	0	75	30	5
2	1	-1	0	125	30	5
3	-1	1	0	75	70	5
4	1	1	0	125	70	5
5	-1	0	-1	75	50	0
6	1	0	-1	125	50	0
7	-1	0	1	75	50	10
8	1	0	1	125	50	10
9	0	-1	-1	100	30	0
10	0	1	-1	100	70	0
11	0	-1	1	100	30	10
12	0	1	1	100	70	10
13	0	0	0	100	50	5
14	0	0	0	100	50	5
15	0	0	0	100	50	5
16	0	0	0	100	50	5
17	0	0	0	100	50	5

A = Amount of HPMC, B = Amount of $NaHCo_3$, C = Amount of Citric Acid
Low = -1, Middle = 0, High = 1

Table III. Composition of formulations

Ingredients	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9	F_{10}	F_{11}	F_{12}	F_{13}	F_{14}	F_{15}	F_{16}	F_{17}	**OF**
Drug (mg)	250	250	250	250	250	250	250	250	250	250	250	250	250	250	250	250	250	250
HPMC (mg)	75	125	75	125	75	125	75	125	100	100	100	100	100	100	100	100	100	80.2
$NaHCO_3$ (mg)	30	30	30	70	70	70	50	50	30	70	30	70	50	50	50	50	50	45.6
Citric Acid (mg)	5	5	5	5	0	0	10	10	0	0	10	10	5	5	5	5	5	3.2
Mg Stearate (mg)	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	8.00
Lactose (mg)	130	80	130	40	95	45	100	55	110	70	100	60	85	85	85	85	85	113
Total (mg)	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500	500

Drug = Ofloxacin

HPMC = Hydroxy propyl methyl cellulose

$NaHCO_3$ = Sodium Bicarbonate

OF = Optimized formulation

Table IV. Floating properties of tablets of all formulations

Batch	Buoyancy lag time (seconds)		Total floating time (hr)	
	Average	SD	Average	SD
F1	19.67	1.53	4.83	0.29
F2	1247	128.58	10.00	0.00
F3	13.67	1.53	3.73	0.25
F4	22.33	2.52	10.00	0.00
F5	37.00	2.65	10.00	0.00
F6	86.67	7.64	10.00	0.00
F7	11.67	1.53	03.17	0.29
F8	16.00	2.00	10.00	0.00
F9	80.33	3.06	10.00	0.00
F10	36.00	3.61	10.00	0.00
F11	25.67	2.08	10.00	0.00
F12	15.33	1.53	04.83	0.29
F13	15.33	0.58	10.00	0.00
F14	15.00	1.00	10.00	0.00
F15	15.00	1.00	10.00	0.00
F16	14.67	0.58	10.00	0.00
F17	15.33	0.58	10.00	0.00
OF	14.35	0.25	10.00	0.00
OFS	25.24	0.23	9.56	0.00

OF = Optimized formulation, OFS = Optimized Formulation after Stability

Response: Total Floating Time (TFT)

Table V. ANOVA for Response Surface Quadratic Model (TFT).

Source	Sum of square	D.F.	Mean Square	F value	Prob>F
Model	71.16	9	7.91	17.56	0.0005
A	29.15	1	29.15	64.74	<0.0001
B	3.47	1	3.47	7.71	0.0274
C	12.5	1	12.5	27.76	0.0012
A	8.11	1	8.11	18	0.0038
B	3.98	1	3.98	8.84	0.0207
C	0.021	1	0.021	0.046	0.8366
AB	0.3	1	0.3	0.67	0.4394
AC	8.5	1	8.5	18.87	0.0034
BC	4.35	1	4.35	9.66	0.0171
Residual	3.15	7	0.045	---	---
Lack of fit	3.15	3	1.05	---	---
Pure error	0	4	0	---	---
Cor total	74.31	16	---	---	---

A = Amount of HPMC, B = Amount of $NaHCo_3$, C = Amount of Citric Acid

DF= degrees of freedom, F – Fischer's ratio

Response: $T_{50\%}$

Table VI. ANOVA for Response Surface Quadratic Model ($T_{50\%}$).

Source	Sum of square	D.F.	Mean Square	F value	Prob>F
Model	134.29	9	14.92	14.04	0.0011
A	49.01	1	49.01	46.12	0.0003
B	4.06	1	4.06	3.82	0.0915
C	22.11	1	22.11	20.81	0.0026
A	14.22	1	14.22	13.38	0.0081
B	10.28	1	10.28	9.67	0.0171
C	0.81	1	0.81	0.76	0.4127
AB	0.49	1	0.49	0.46	0.5189
AC	9	1	9	8.47	0.0226
BC	23.52	1	23.52	22.14	0.0022
Residual	7.44	7	1.06	---	---
Lack of fit	7.24	3	2.41	---	---
Pure error	0.2	4	0.05	---	---
Cor total	141.73	16	---	---	---

A = Amount of HPMC, B = Amount of $NaHCo_{3,}$ C = Amount of Citric Acid

DF= degrees of freedom, F – Fischer's ratio**,** T 50 % = Time Required to Release 50% of drug

Response: % $CR_{10\ hrs}$

Table VII. ANOVA for Response Surface Quadratic Model (% $CR_{10\ hrs}$).

Source	Sum of square	D.F.	Mean Square	F value	Prob>F
Model	3441.68	9	382.41	22.7	0.0002
A	1497.14	1	1497.14	88.86	<0.0001
B	55.76	1	55.76	3.31	0.1117
C	722	1	722	42.86	0.0003
A	239.88	1	239.88	14.24	0.007
B	233.57	1	233.57	13.86	0.0074
C	50.61	1	50.61	3	0.1267
AB	1.14	1	1.14	0.068	0.8018
AC	159.52	1	159.52	9.47	0.0179
BC	476.99	1	476.99	28.31	0.0011
Residual	117.93	7	16.85	---	---
Lack of fit	110.23	3	36.74	---	---
Pure error	7.7	4	1.92	---	---
Cor total	3559.61	16	---	---	---

A = Amount of HPMC, B = Amount of $NaHCo_{3,}$ C = Amount of Citric Acid,

DF= degrees of freedom, F – Fischer's ratio**,** %CR 10 hr = Percentage Cumulative Release at 10Hr,

Table VIII. Dissolution data of all formulations [including stability batch (OFS)]

Time Hrs	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16	F17	**OF**	**OFS**
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	47.91	9.97	70.04	3.21	4.29	3.58	83.59	2.87	6.57	1.67	12.17	69.35	9.91	11.39	7.71	8.48	11.71	09.80	08.23
2	61.07	17.53	86.90	10.34	8.57	11.15	92.58	9.14	15.75	5.42	19.70	87.13	15.70	16.67	10.94	12.39	17.86	18.82	12.25
3	69.07	25.14	94.04	19.53	16.86	19.74	95.53	16.65	23.43	9.35	24.57	90.92	20.54	21.10	16.05	19.39	22.01	28.36	23.32
4	77.57	32.17	96.63	27.11	23.78	25.99	96.66	25.16	32.30	16.18	32.17	94.17	26.60	28.66	23.29	27.23	28.47	37.0	39.6
5	83.02	38.01	97.78	32.56	29.88	29.88	98.15	31.65	38.80	22.75	39.30	96.29	32.85	34.51	29.25	34.36	37.48	45.61	46.2
6	89.36	46.60	98.73	40.99	38.60	37.12	97.93	38.99	44.88	29.76	45.00	97.48	40.49	40.12	37.08	41.78	44.59	53.89	59.3
7	91.73	53.53	99.48	47.81	47.41	43.25	96.63	45.50	50.48	38.28	50.52	98.99	46.56	47.95	44.64	48.54	51.54	61.74	65.23
8	95.12	58.71	99.65	54.97	56.42	49.06	99.23	53.16	55.81	45.12	55.76	99.89	54.79	54.96	52.86	58.19	58.88	69.69	70.2
9	98.47	64.90	99.82	61.76	60.83	53.49	99.00	58.39	62.79	52.96	61.81	99.91	62.14	63.48	59.14	64.03	65.34	76.80	75.21
10	99.56	69.26	99.93	67.49	69.91	59.19	99.73	63.75	67.93	57.35	66.90	99.97	67.85	67.92	68.49	70.91	70.15	82.37	80.1

OF = Optimized formulation, OFS = Optimized Formulation after Stability

Table IX. Dissolution release kinetic study of all formulations [including stability batch (OFS)]

Batch	Zero order		Higuchi		Korsmeyer Peppas (Kp)		
	K_0	r^2	K_H	r^2	n	r^2	K_m
F1	12.789	0.3335	35.193	0.9258	0.324	0.9952	48.65
F2	7.376	0.9880	19.331	0.9133	0.856	0.9992	9.84
F3	13.187	-0.6216	39.456	0.5383	0.138	0.8341	76.09
F4	6.765	0.9951	17.450	0.8356	1.285	0.9842	3.96
F5	6.695	0.9859	17.148	0.7977	1.243	0.9966	4.10
F6	6.051	0.9957	15.710	0.8674	1.166	0.9791	4.50
F7	13.971	-0.9879	36.660	0.3385	0.066	0.8534	86.66
F8	6.417	0.9925	16.520	0.8261	1.328	0.9875	3.45
F9	7.130	0.9903	18.690	0.9015	0.989	0.9907	7.45
F10	5.393	0.9586	13.650	0.7377	1.558	0.9977	1.76
F11	7.130	0.9730	18.789	0.9376	0.751	0.9965	11.65
F12	13.947	-0.5441	39.256	0.5770	0.146	0.8911	74.59
F13	6.801	0.9963	17.684	0.8716	0.857	0.9873	8.83
F14	6.913	0.9929	18.033	0.8859	0.806	0.9816	9.92
F15	6.473	0.9896	16.640	0.8134	1.000	0.9778	6.24
F16	7.040	0.9974	18.228	0.8468	0.975	0.9875	7.26
F17	7.282	0.9923	19.015	0.8926	0.814	0.9827	10.33
OF	8.66	0.9942	28.12	0.9406	0.9336	0.9992	0.99
OFS	8.33	0.9956	27.22	0.9123	0.9456	0.9991	0.98

OF = Optimized formulation, OFS = Optimized Formulation after Stability

K_o= Zero order rate constant, K_p= Kosrsmeyer Peppas rate constant,

K_H = Higuchi rate constant, n = Diffusion concentration,

R^2 = Regression coefficient, Km = Mean of constants.

Response: Diffusion coefficient (n)

Table X. ANOVA for Response Surface Quadratic Model (Diffusion coefficient).

Source	Sum of square	D.F.	Mean Square	F value	Prob>F
Model	3.01	9	0.33	18.91	0.0004
A	1.03	1	1.03	57.94	0.0001
B	5.35E-003	1	5.35E-003	0.30	0.5993
C	0.89	1	0.89	50.16	0.0002
A2	0.024	1	0.024	1.34	0.2856
B2	0.11	1	0.11	6.45	0.0386
C2	0.077	1	0.077	4.36	0.0753
AB	0.095	1	0.095	5.34	0.0541
AC	0.45	1	0.45	25.33	0.0015
BC	0.34	1	0.34	19.47	0.0031
Residual	0.12	7	0.018	---	---
Lack of Fit	0.091	3	0.030	3.64	0.1223
Pure Error	0.033	4	8.31E-003	---	---
Cor Total	3.14	16	---	---	---

A = Amount of HPMC, B = Amount of $NaHCo_{3,}$ C = Amount of Citric Acid,

DF= degrees of freedom, F – Fischer's ratio**,**

Table XI. Constraints

Name	Goal	Lower Limit	Upper Limit
Amount of HPMC	minimize	75	125
Amount of NaHCo3	minimize	30	70
Amount of Citric acid	maximize	0	10
TFT	maximize	4.17	10
%CR 10 hr	maximize	57.37	100
T 50 %	Is target = 5.00	0.6	8.6
Diffusion coefficient (n)	Is target = 1.00	0.066	1.558

TFT= Total floating time, %CR 10 hr = % Cumulative Release at 10Hr,

T 50 % = Time Required to Release 50% of drug

Table XII. Comparision between observed values and predicted values of optimised formulation

Response	Observed	Predicted	% Error
Total floating time	10 hours	8.8	12.00
$T_{0.5}$	5.3 hours	5.0	5.66
% CR_{10hrs}	82.37	80.22	2.62
Diffusion coefficient(n)	0.93	0.91	2.15

Table XIII. The design and response summary data

Std	Factors			Response			
	A: Amt of HPMC	**B: Amt of $NaHCo_3$**	**C: Amt of Citric Acid**	**TFT hrs**	**%CR_1 0 hr**	**$T_{50\%}$ hrs**	**n**
1	75.00	30.00	05.00	5.83	99.56	1.2	0.324
2	125.00	30.00	05.00	10.00	69.26	6.4	0.856
3	75.00	70.00	05.00	04.73	99.93	0.7	0.138
4	125.00	70.00	05.00	10.00	67.49	7.3	1.285
5	75.00	50.00	00.00	10.00	69.91	7.2	1.243
6	125.00	50.00	00.00	10.00	59.19	8.2	1.166
7	100.00	50.00	10.00	04.17	99.73	0.6	0.066
8	125.00	50.00	10.00	10.00	63.75	7.6	1.328
9	75.00	30.00	00.00	10.00	67.93	6.8	0.989
10	75.00	70.00	00.00	10.00	57.35	8.6	1.558
11	75.00	30.00	10.00	10.00	66.90	8.6	0.751
12	75.00	70.00	10.00	05.83	99.93	0.7	0.146
13	75.00	50.00	05.00	10.00	67.85	7.4	0.857
14	75.00	50.00	05.00	10.00	67.92	7.3	0.806
15	75.00	50.00	05.00	10.00	68.49	7.6	1.000
16	75.00	50.00	05.00	10.00	70.91	7.2	0.975
17	75.00	50.00	05.00	10.00	70.15	7.0	0.814
OF	80.2	45.6	3.2	10.00	72.31	7.6	0.924
OFS	80.2	45.6	3.2	9.22	70.1	7.3	0.921

OF = Optimized formulation, OFS = Optimized Formulation after Stability

TFT= Total floating time, %CR 10 hr = Percentage Cumulative Release at 10Hr,

T 50 % = Time Required to Release 50% of drug, n = Diffusion concentration