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Formulation and optimization of gastric floating matrix tablets of Gatifloxacin with combination of polymers using Box-Behnken experimental design

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Abstract

The purpose of the present study was to develop an optimized gastric floating drug delivery system (GFDDS) containing gatifloxacin as a model drug using Box-Behnken design. A 3-factor, 3-level Box-Behnken design was used to derive a second order polynomial equation and construct contour plots to predict responses. The independent variables selected were conc. of compritol ATO 888 (X_1), conc. of poloxamer 188 (X_2) and conc. of chitosan (X_3). Batches were prepared by wet granulation method and evaluated for Floating lag time (FLT), Total floating time (TFT) and time required to release 50% of the drug (t_{50}) as dependent variables. Compritol ATO 888 containing tablets were found to be significant for floating properties. Poloxamer 188 had a negative effect on floating properties but was found helpful in controlling the release rate of the drug. No significant effect of chitosan on floating properties was observed but it was important for gel formation. The quadratic mathematical model developed could be used to predict formulations with desired release and floating properties. The transformed values of the independent variables and dependent variables were subjected to multiple regressions to establish a full-model second-order polynomial equation. Contour plots as well as response surface plots were constructed to show the effects of X_1, X_2 and X_3 on the FLT, TFT and t_{50}. A model was validated for accurate prediction of the FLT, TFT and t_{50} by performing checkpoint analysis. The computer optimization process, contour plots and response surface plots predicted at the conc. of independent variables X_1, X_2, and X_3 (25.25 %, 13.62% and 15% respectively), for maximized response of TFT. The Box-Behnken design demonstrated the role of the derived equation and contour plots with response surface plots in predicting the values of dependent variables for the preparation and optimization of gatifloxacin gastric floating matrix tablet.

Keywords: Gatifloxacin, floating, Box-Behnken design, total floating time (TFT), compritol ATO 888.

INTRODUCTION

The real challenge in the development of a controlled drug delivery system is not just to sustain the drug release but also to prolong the presence of the dosage form in the stomach or the upper small intestine until all the drug is completely released in the desired period of time [1–2]. The residence of a drug delivery system in the upper part of the gastrointestinal tract (GIT) can be accomplished by several drug delivery systems, such as intragastric floating systems [3-5], swelling and expandable systems [6], bioadhesive systems [7], modified shape systems [8], high density systems [9], delayed gastric emptying systems [10] and low density super porous systems [11]. FDDS, also called hydrodynamically balanced system, is an effective technology to prolong the gastric residence time in order to improve the bioavailability of the drug. This technology is suitable for drugs with an absorption window in the stomach or in the upper part of the small intestine, drugs acting locally in the stomach and for drugs that are poorly soluble or unstable in the intestinal fluid. FDDS have a bulk density lower than the gastric fluid and thus remain buoyant in the stomach, without affecting the gastric emptying rate for a prolonged period of time. While the system is floating on the gastric contents, the drug is released slowly [12]. Gatifloxacin, the model drug for this study, is an 8-methoxyfluoroquinolone with *in vitro* activity against a wide range of gram-negative and gram-positive microorganisms. The antibacterial action of gatifloxacin results from inhibition of DNA gyrase and topoisomerase IV, essential enzymes that are involved in the replication, transcription, and repair of bacterial DNA. The recommended adult oral dosage of gatifloxacin is 200mg twice daily or 400mg daily. The solubility of the compound is pH dependent. The maximum aqueous solubility (40-60 mg/ml) occurs at a pH range of 2 to 5 [13, 14]. The bioavailability of gatifloxacin is 96%. It is one of the drugs with absorption window, so its primary site of absorption is the stomach region. Research is also going on the various delivery approaches for gatifloxacin. Motwani *et al* [15] has reported nanoparticles for ophthalmic delivery containing gatifloxacin. Amal *et al* [16] has also reported gatifloxacin biodegradable implant for treatment of experimental osteomyelitis. Recently, gatifloxacin is proved to be one of the potential drugs against H.pylori infection, responsible for duodenal ulcers and various cytotoxic complications. H.pylori resides mainly in stomach region, specifically in the sub-region of the mucous layer in stomach [17]. It is also reported that a stomach specific locally targeted dosage form would be more effective against H.pylori compared to the conventional one [17]. So it demands prolonged and constant drug conc. at that particular site to eradicate the infection. This leads to the formulation of clinically acceptable sustained-release dosage forms of gatifloxacin. The gastroretentive drug delivery systems can be retained in the stomach and assist in improving the oral sustained delivery of drugs that have an absorption window in a particular region of the gastrointestinal tract. The local delivery of gatifloxacin by this approach will also promote a fast and effective eradication of H.pylori rather than a conventional tablet containing gatifloxacin.

In the work reported here, a Box-Behnken design [18] was used to optimize floating matrix tablet containing gatifloxacin. The independent variables selected were Compritol ATO 888 (X_1), poloxamer 188 (X_2) and chitosan (X_3). Batches were prepared by wet granulation method and evaluated for Floating lag time (FLT), total floating time (TFT) and time required to release 50% of the drug (t_{50}) as dependent variables.

MATERIALS AND METHODS

Materials:

Compritol ATO 888 was a gift from Gattefosse (St Priest, Cedex, France). Poloxamer 188 was gifted BASF India Limited (Mumbai, India). Chitosan was gifted by Mahtani chitosan pvt. Ltd. (Veraval, India). Gatifloxacin was purchased from West-Coast Pharmaceuticals Ltd. (Ahmedabad, India). Concentrated hydrochloric acid (HCL) was kindly supplied by Purvi Chemicals (Ahmedabad, India). All other chemicals were of analytical grade.

Methods:

Fabrication of gatifloxacin floating tablets

Gatifloxacin was mixed with the required quantity of Compritol ATO 888, poloxamer 188 and chitosan, sodium bicarbonate and lactose with a spatula in a mortar for 5 min. Isopropyl alcohol was added drop wise until a suitable mass for granulation was obtained. Then the wet mass was granulated through a 10 mesh sieve. The granules were dried at room temperature (35 °C) for 1 h and then blended with 2% talc, 1% magnesium stearate and compressed on a 8-station rotary tablet compression machine (Rimek, India) using a 12.5-mm standard flat-face punch.

Box-Behnken experimental design

A Box-Behnken statistical design with 3 factors, 3 levels, and 15 runs was selected for the optimization study. The experimental design consists of a set of points lying at the midpoint of each edge and the replicated center point of the multidimensional cube. The independent and dependent variables are listed in Table I. The polynomial equation generated by this experimental design (using Sigma Plot 11) is as follows:

$$Y_i = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_3 + b_{12} X_1 X_2 + b_{13} X_1 X_3 + b_{23} X_2 X_3 + b_{11} X_1^2 + b_{22} X_2^2 + b_{33} X_3^2 \quad \textbf{(1)}$$

where Yi is the dependent variable, b_0 is the intercept, b_1 to b_{33} are the regression coefficients, and X_1, X_2 and X_3 are the independent variables that were selected from the preliminary experiments.

In vitro buoyancy studies

The *in vitro* buoyancy was determined by floating lag times according to the method described by Rosa *et al*. [19]. The tablets were placed in a 100-mL beaker containing 0.1 mol L^{-1} HCl. The time required for the tablet to rise to the surface and float was taken as the floating lag time (FLT). The experiments were conducted in triplicate.

In vitro dissolution studies

The release rate of gatifloxacin from floating matrix tablets (n = 3) was determined using the Dissolution Testing Apparatus II (paddle method). The dissolution test was performed using 900 ml of 0.1 mol L^{-1} HCl, at 37±0.5 °C and 50 rpm. A 5-mL sample was withdrawn from the dissolution apparatus hourly for 24 h, and the samples were replaced with fresh dissolution medium.

The samples were filtered through a 0.45µm membrane filter and diluted to a suitable concentration with 0.1 mol L^{-1} HCl. Absorbance of these solutions was measured at 292 nm wavelength (λmax) using a Shimadzu UV-1700 UV/Vis double-beam spectrophotometer (Japan). Cumulative drug release was calculated using the equation generated from Beer Lambert's calibration curve in the linearity range of 0-25 µg mL^{-1}. FLT and TFT of the tablets were measured during dissolution studies.

Statistical analysis

Statistical analysis of the Box-Behnken design batches was performed by multiple regression analysis using Microsoft Excel. To evaluate the contribution of each factor with different levels to the response, the two-way analysis of variance (ANOVA) was performed using the DESIGN EXPERT 8.0.1 (STAT-EASE) demo version software. To graphically demonstrate the influence of each factor on the response, the response surface plots were generated using the DESIGN EXPERT 8.0.1 (STAT-EASE) demo version software.

Checkpoint Analysis

A checkpoint analysis was performed to confirm the role of the derived polynomial equation and contour plots in predicting the responses. Values of independent variables were taken at 3 points, 1 from each contour plot, and the theoretical values of TFT were calculated by substituting the values in the polynomial equation. Floating matrix tablets were prepared experimentally at 3 checkpoints and evaluated for the responses.

Optimization Data Analysis

The computation for optimized formulation was carried using software, DESIGN EXPERT 8.0.1 (STAT-EASE). The optimized formulation was obtained by applying constraints (goals) on dependent (response) and independent variables (factors). Constraints for responses and factors are shown in Table 2.

The models were evaluated in terms of statistically significant coefficients and R^2 values. Various feasibility and grid searches were conducted to find the optimum parameters. Various 3-D response surface graphs were provided by the Design Expert software. The optimized checkpoint formulation factors were evaluated for various response properties. The resultant experimental values of the responses were quantitatively compared with the predicted values to calculate the percentage prediction error.

All batches contain 400 mg of gatifloxacin, 10% sodium bicarbonate, 2% talc, 1% Magnesium stearate and a sufficient quantity of lactose to adjust the average mass of tablets to 650 mg.

Table I. Variables and Observed Responses in Box–Behnken Design for compression coated tablets

Batch	Independent variables			Dependent variables			Levels used, Actual (Coded)		
	X_1	X_2	X_3	Y_1	Y_2	Y_3	Low (-1)	Medium (0)	High (1)
S1	-1	1	0	1±0.5	3.3±0.1	13.1±0.1			
S2	0	-1	-1	8±2	10.8±0.4	12.5±0.1			
S3	1	-1	0	3±1	24.8±0.5	13.3±0.0			
S4	-1	0	-1	10±4	5±0.2	12±0.2			
S5	-1	0	1	6±2	6.1±0.1	11.9±0.1			
S6	0	-1	1	2±0.5	24.8±0.4	14.8±0.2			
S7	1	0	1	25±4	6.4±0.3	14.7±0.3			
S8	-1	-1	0	3±1	8.8±0.4	12±0.0			
S9	0	0	0	3±1.5	3.3±0.4	15.8±0.1			
S10	1	1	0	14±5	5.2±0.1	12±0.2			
S11	0	1	-1	32±5	4.4±0.1	12.8±0.1			
S12	0	1	0	14±3	5.7±0.2	11.1±0.4			
S13	1	0	-1	2±0.5	24.8±0.3	11.3±0.2			
S14	0	0	0	4±1.5	4.6±0.1	14.5±0.3			
S15	0	0	0	3.5±1	3.6±0.2	15±0.2			
Independent variables									
X_1 = Conc. of Compritol ATO 888 (%)							10	20	30
X_2 = Conc. of poloxamer 188 (%)							5	10	15
X_3 = Conc. of chitosan (%)							15	25	35
Dependent variables Y_1 = Floating lag time (FLT) (S) Y_2= total floating time (TFT) (h) Y_3= time required to release 50% of the drug (t_{50}) (h)									

Table II Constraints for optimization

Name	Goal	Upper limit	Lower limit
Conc. of Compritol ATO 888 (%)	In range	30	10
Conc. of poloxamer 188 (%)	In range	15	5
Conc. of chitosan (%)	In range	35	15
Floating lag time (FLT) (S)	Target - less than 60 s.		
total floating time (TFT) (h)	Target - between 20-24 h.		
time required to release 50% of the drug (t_{50}) (h)	Target - between 10-12 h		

RESULTS AND DISCUSSION

In the present investigation, combinations of three polymers were studied using the Box Behken design. The mathematical models developed for all the dependent variables using statistical analysis software are shown in Equations (2-4):

Floating lag time (FLT) = $4.85 + 3.00X_1 + 6.67\ X_2 + 0.11X_3 + 6.75\ X_1X_3 + 8.26X_3^2$ --- (2)

Total floating time (TFT) = $8.91 + 4.75\ X_1 - 7.32X_2 - 1.76X_3 - 3.52X_1X_2 - 4.88X_1X_3 - 6.19X_2X_3$--- (3)

Time required to release 50% of the drug (t_{50}) = $14.78 + 0.29X_1 - 0.57X_2 + 0.61X_3 - 0.60X_1X_2 + 0.87X_1X_3 - 0.75X_2X_3 - 1.25X_1^2 - 1.16X_2^2 - 0.80X_3^2$ --- (4)

The floating lag time for all tablets was found to be below 60 s regardless of the content of polymers used (Table I), indicating insignificant effect of the concentration of polymers. Lower value of the correlation coefficient (Eq. 2) clearly indicates that the response is independent of the factors studied. This was due to evolution and entrapment of carbon dioxide inside the hydrated polymeric matrices, resulting from the interaction between the gas generating agent ($NaHCO_3$) and dissolution medium (0.1 mol L^{-1} HCl, pH 1.2) which led to lowering of the density of matrices enabling the tablets to float.

The results of TFT and t_{50} showed wide variations (Table I). From the results of multiple regression analysis, it was found that the dependent variables, TFT and t_{50}, are strongly dependent on the independent variables ($p < 0.05$). The correlation coefficients indicate a good fit. Polynomial equations (Eq. 3 and 4) can be used to draw a conclusion after considering the magnitude of the coefficient and the mathematical sign it carries (positive or negative). As the amount of poloxamer 188 increased, TFT decreased; this may be due to high affinity of poloxamer 188 toward water, which promotes water penetration into tablet matrices, leading to increased density. As the amount of compritol ATO 888 increased, TFT increased; this is because of increased gel strength of matrices, which prevents escape of evolved carbon dioxide from matrices, leading to decreased density. As the amount of chitosan increased, TFT decreased; this is because of the poor gelling strength of chitosan. This effect of polymer concentration is reflected in formulations S3, S6 and S13 (shown in Table I). As the amount of compritol ATO 888 and poloxamer 188 increased, sustained release of gatifloxacin was observed. Since gatifloxacin is freely soluble in acidic media, compritol ATO 888, because of its lipid nature, control and sustain the release of the drug. Due to high affinity of poloxamer towards water, water penetration into tablet matrices increased, leading to solubilization and release of gatifloxacin.

Table III. Checkpoint batches with predicted and measured TFT

Batch code	X_1	X_2	X_3	TFT (h)	
				Measured [a]	Predicted
1	0	-0.5	0.5	20.8±0.4	20.15
2	0.5	0	-0.5	18.8±0.3	19.05
3	-0.5	0.5	0	2.3±0.1	2.45

[a] *Mean ±SD, n = 3.*

Three checkpoint batches were prepared and evaluated for TFT, as shown in Table III. Results indicate that the measured TFT values were as expected. When measured TFT values were compared with predicted TFT values using Student's t-test, the differences were found to be not significant. Thus, we can conclude that the obtained mathematical equation is valid for predicting TFT.

To demonstrate graphically the effect of the amount of compritol ATO 888, Poloxamer 188 and chitosan, the counter and response surface plots were generated for the dependent variables FLT, TFT and t_{50}

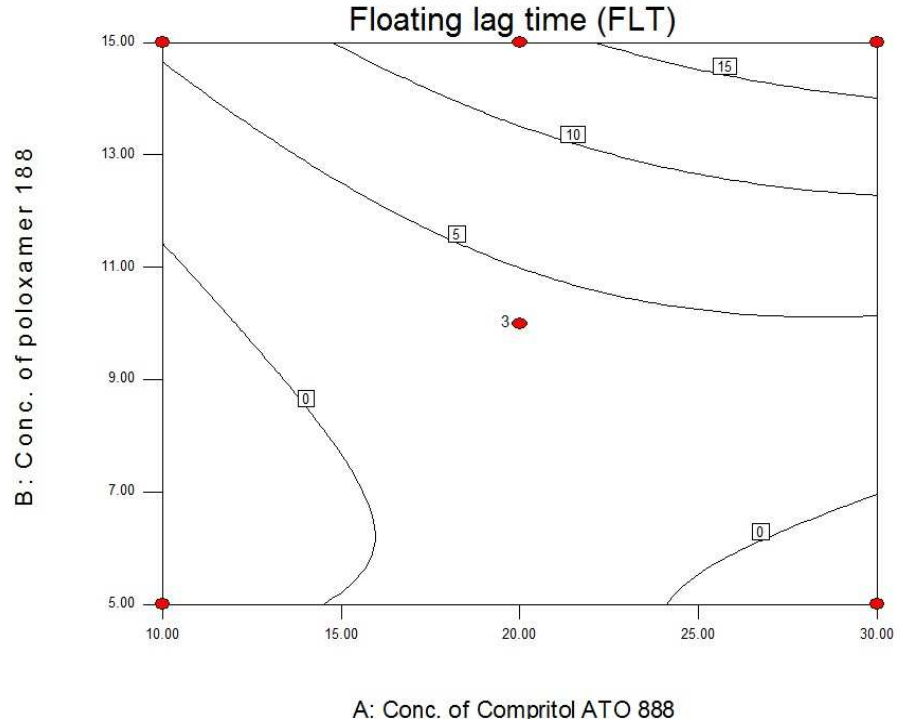

Fig 1 (a)

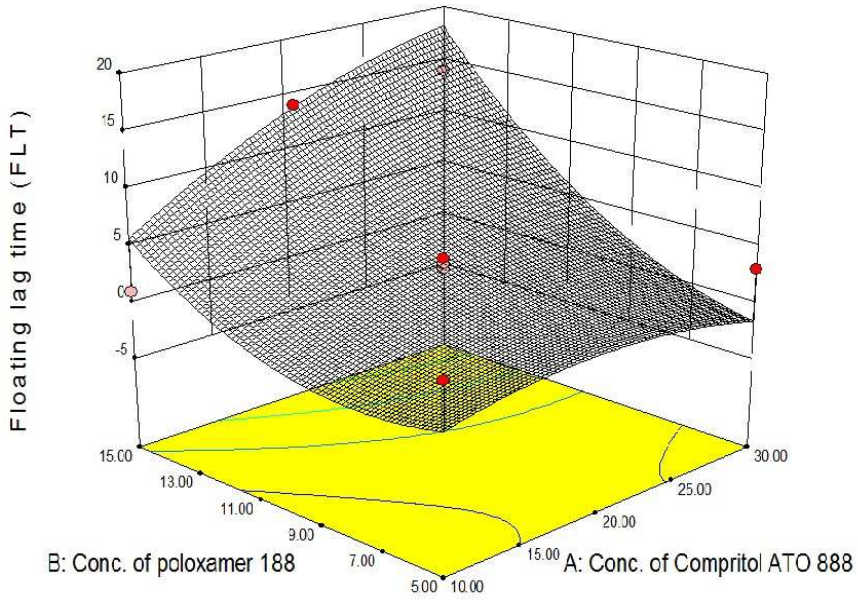

Fig 1 (b)

Fig. 1 (a) and 1(b) counter plot and response surface plot for the effect of polymer amount on (FLT)

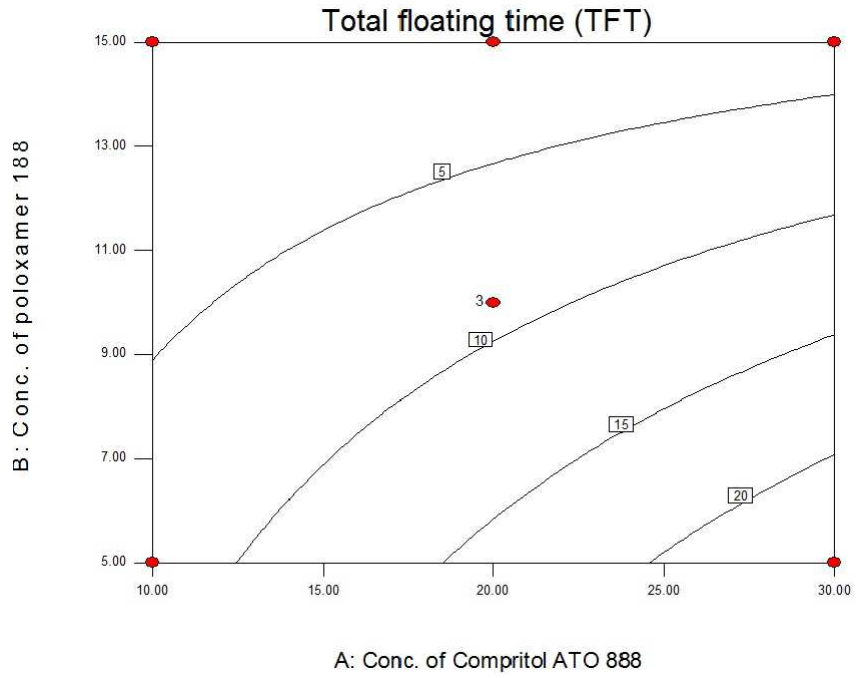

Fig 2 (a)

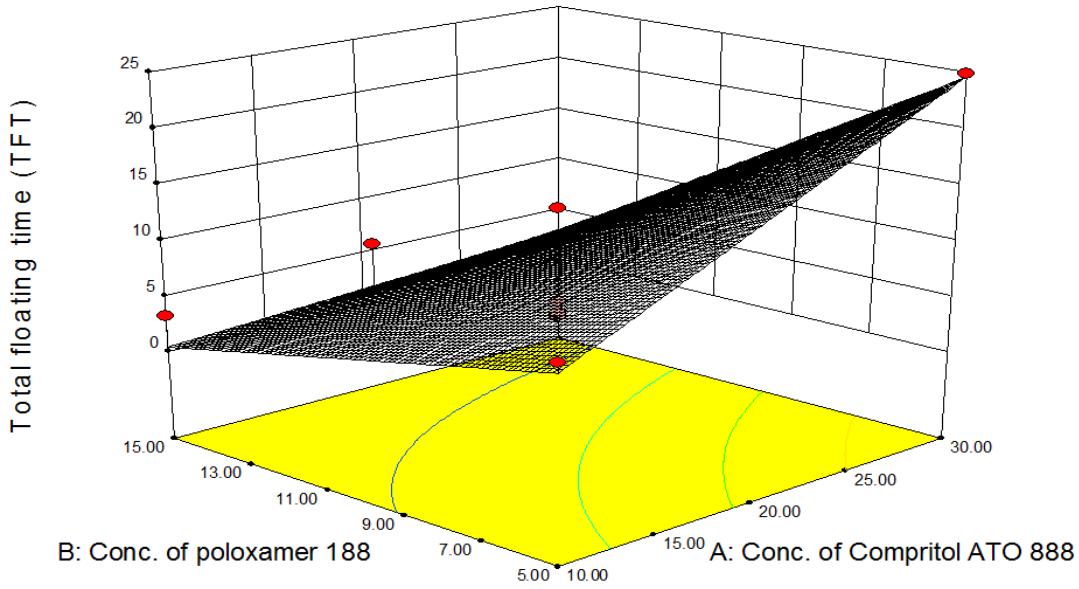

Fig 2 (b)

Fig. 2 (a) and 2(b) counter plot and response surface plot for the effect of polymer amount on TFT

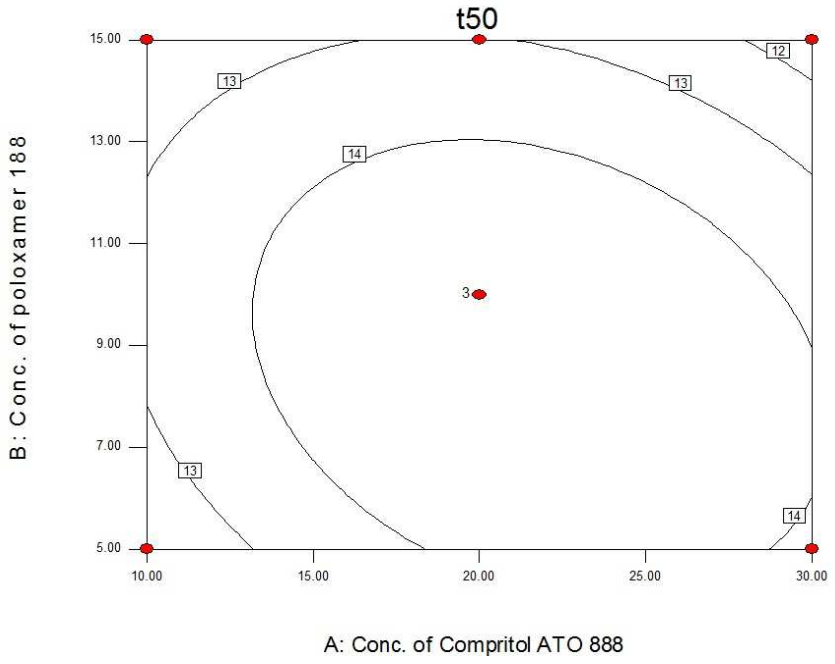

Fig 3 (a)

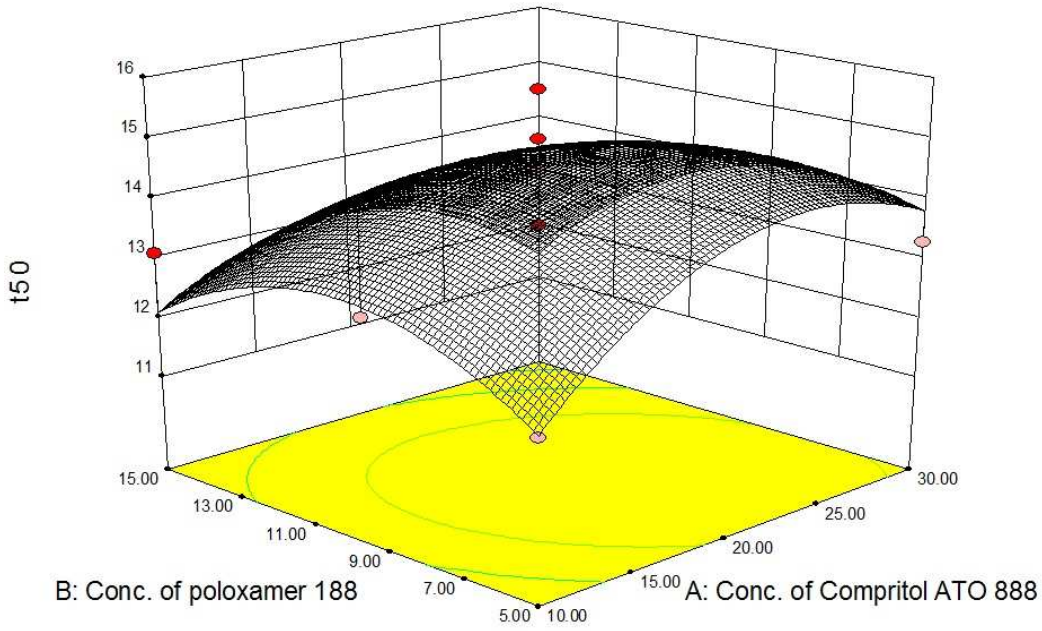

Fig 3 (b)

Fig. 3 (a) and 3 (b) counter plot and response surface plot for the effect of polymer amount on t_{50}

Formulation Optimization

For the optimization of floating tablets of gatifloxacin, constraints were fixed for all factors and response (Table 2). Constraints were set according to formulation of floating granules using minimum amt of excipients, which would give desired response values. In the present study our aim was floating lag time should be less than 60 s., floating time should be 22 hrs. and t_{50}% should be 12 hrs. In optimization (Fig. 4) desirability 0.984 indicated optimum formulation was achieved at 25.25 % of X_1, 13.62% of X_2 and 15% of X_3. Validation of optimization technique done by preparing check-point batch and response were evaluated. The responses value observed in checkpoint batch was very near to optimized batch.

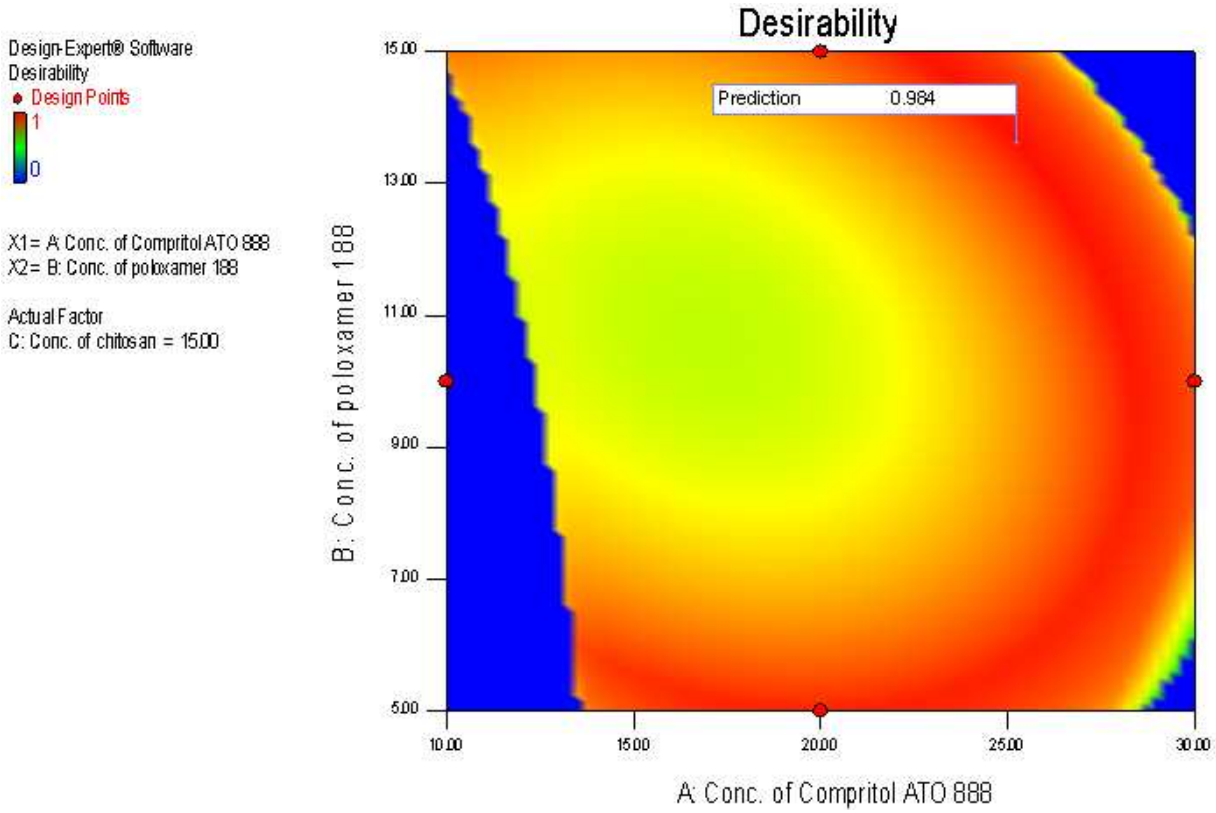

Fig 4 Countour plot for optimization

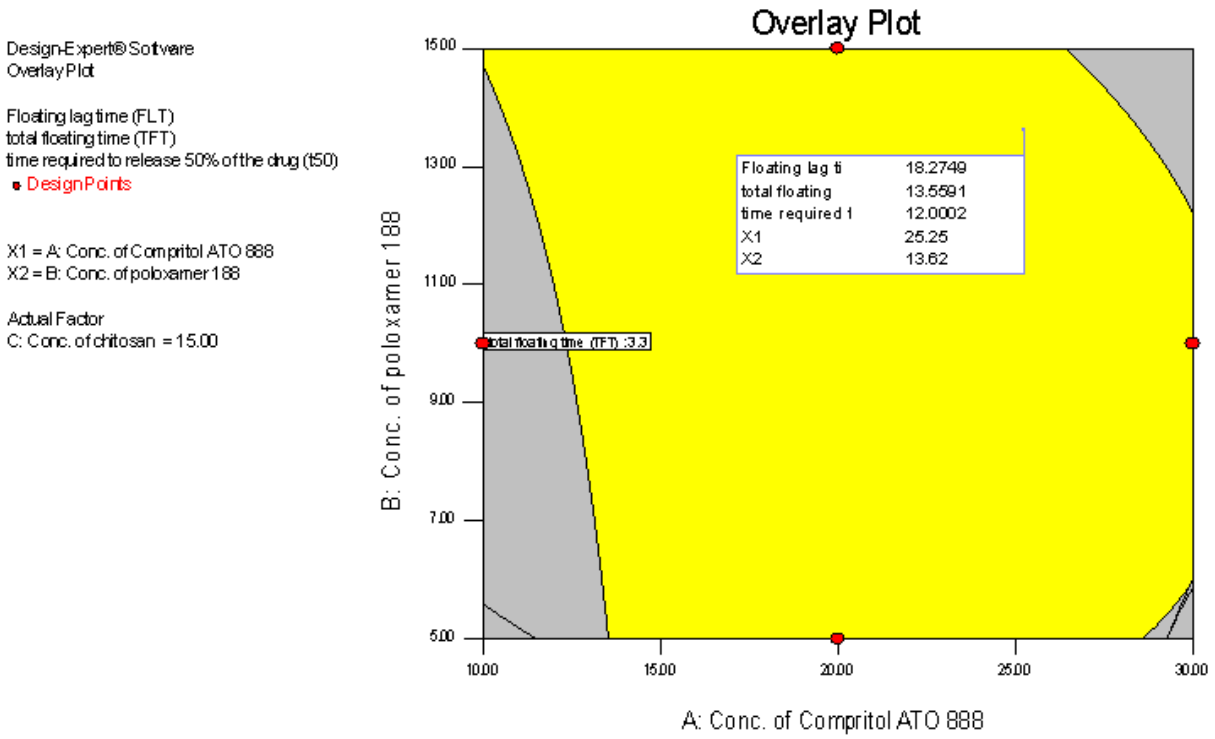

Fig 5 Overlay plot for optimization

CONCLUSION

Compritol ATO 888, poloxamer 188 and chitosan significantly affect FLT, TFT and t_{50} of the formulated GFDDS. When they are used in combination for developing GFDDS, high to moderate amount of Compritol ATO 888, low to moderate amount of poloxamer 188 and low to high amount of chitosan is to be used to achieve the desired FLT, TFT and release profile required for once daily formulations.

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