ORIGINAL ARTICLE

Application of Full 3^2 Factorial Design in Formulation and Optimization of Hydrodynamically Balanced System of Ketorolac Tromethamine

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Abstract

Introduction: The primary objective of this research work was to design a hydrodynamically balanced system (HBS) of ketorolac tromethamine based on the combination of swelling and effervescence mechanism that would result in minimum floating lag time (FLT), remain buoyant for extended period, and sustain the drug release for 12 h. **Materials and Methods:** HBS tablets of ketorolac tromethamine were prepared by wet granulation method using 3^2 full factorial design, where amount of hydroxypropyl methylcellulose K4M (X_1) and sodium bicarbonate ($NaHCO_3$) (X_2) was taken as independent variables. The responses studied were Y_1 (hardness), Y_2 (FLT), Y_3 total floating time (TFT), Y_4 (swelling index [SI] in pH1.2), and Y_5 ($t_{80\%}$) by plotting response surface graph and contour plots. **Results:** The FLT of prepared batches was in the range of 35.24 ± 0.04–116 ± 0.06 s and the minimum FLT was shown by OF-9 (35.24 s) batch. The TFT of all batches showed buoyancy and intactness for 24 h. The swelling in pH 1.2 at 8 h ranged between 47.80 ± 0.89 and 54.33 ± 0.11 with a significant difference ($P < 0.0001$). *In vitro* drug release study revealed that more than 95% of drug was released in 12 h and OB-2 showed 10 h to release 80% of the drug. The analytical characterization revealed the compatibility of drug with excipient and polymers. The stability studies performed for 6 months for OF-7 and OF-8 batches showed no change in physicochemical properties of drug and there was no significant difference ($P < 0.05$) in drug release. The response surface graph and contour plots showed the effect of variables on responses such as hardness (Y_1), SI (Y_4), and time taken for 80% of drug release ($t_{80\%}$)(Y_5) which were found to be positively influenced by X_1 and ANOVA for response surface quadratic model was found to be significant ($P < 0.0001$). The FLT (Y_2) and TFT (Y_3) were found to be affected by X_2 and ANOVA for the response surface quadratic model was found to be significant ($P < 0.05$). **Conclusion:** From all the formulations, OF-8 showed ideal results in the form of hardness, FLT, TFT, SI, and time taken for 80% of drug release ($t_{80\%}$) and stability based on which it can be selected for *in vivo* studies.

Key words: ANOVA, factorial design, floating lag time, ketorolac tromethamine, response surface methodology, total floating time

INTRODUCTION

The oral bioavailability of many drugs is restricted by poor physicochemical properties or absorption in a well-defined region of the gastrointestinal tract (GIT) known as the "absorption window." Prolonged stomach retention enhances the bioavailability, decreases drug waste, and increases solubility for drugs that are less soluble in high pH environments.[1] Hydrodynamically balanced system (HBS), low-density systems, raft systems incorporating alginate gel, bioadhesive or mucoadhesive systems, high-density systems, super porous hydrogel, and magnetic systems are currently used to formulate a successful gastroretentive drug delivery system. The floating dosage formulations have been the most used.[2]

HBS is one of the technologies utilized to provide adequate drug bioavailability by gastric retention.[3] This technique

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is useful for drugs with absorption windows in the stomach or upper small intestine.[4] Because HBS has a lower bulk density than stomach content, it generates buoyancy without influencing gastric emptying rate for an extended length of time, allowing the medicine to be released slowly and at the optimal pace from the system.[5,6] The floating feature is obtained by the formation of gas bubbles, and these buoyant systems make use of matrices made with swellable polymers such as hydroxypropyl methylcellulose K4M (HPMC K4M), HPMC K15M, and effervescent components such as sodium bicarbonate, citric acid, and tartaric acid.[7]

Ketorolac tromethamine is available in market as immediate-release tablet for the treatment of acute pain and inflammation.[5] Hence, for the treatment of chronic and moderate pain, a HBS formulation was prepared. Ketorolac tromethamine is freely soluble in water; hence, release-retarding polymers such as HPMC K4M and K15M play an important role in controlling the release of ketorolac tromethamine from the formulation. The HBS formulations can be achieved by incorporating gas-generating agent such as $NaHCO_3$.

The primary objective of this research work was to design an HBS of ketorolac tromethamine based on the combination of swelling and effervescence mechanism that would result in minimum floating lag time (FLT), remain buoyant for extended period, and sustain the drug release for 12 h.

A 3^2 full factorial design[8] was applied in which the amount of HPMC K4M (X_1) and $NaHCO_3$ (X_2) was taken as independent variables. The responses studied were Y_1 (hardness), Y_2 (FLT), Y_3 total floating time (TFT), Y_4 (swelling index [SI]), and Y_5 ($t_{80\%}$) by plotting response surface graph.

MATERIALS AND METHODS

Materials

Model drug ketorolac tromethamine was purchased from Yarrow Pharma, Mumbai, India. HPMC K4M and HPMC K15 M (50 centipoise viscosity in a 2% w/v solution at 20°C) were kindly supplied by Vishal Chem, Navi Mumbai. Sodium bicarbonate, ethyl cellulose, polyvinylpyrrolidone (PVP), isopropyl alcohol (IPA), magnesium stearate, lactose, and talc were procured from Loba Chemie Pvt. Ltd, Mumbai, India. All other solvents used were of analytical grade and purchased from Merck, Mumbai, India.

Methods

Preparation of HBS of ketorolac tromethamine

All the ingredients sufficient for a batch of 100 tablets as per the formula shown in Table 1 were utilized.[9] Ketorolac tromethamine (equivalent to 10 mg) was accurately weighed and mixed with different HPMC K4M and $NaHCO_3$ ratios. The other ingredients such as ethylcellulose, HPMC K15M, and lactose were added in geometric proportion. The 5% w/v PVP: IPA solution used as a binder is prepared and added drop by drop to the powder mixture until a cohesive mass is formed. The mass of the powder mixture was passed through #12 mesh to obtain granules. A hot air oven was used to dry the granules at 60°C for 10 min, passed through # 20 mesh, and collected on #40 mesh to get uniform particle size. The talc and magnesium stearate were added to dried granules and prepared granules were compressed into tablets using 16-mm punch with a 16-station rotary tablet compression machine (Cadmach, Ahmedabad, India).

Table 1: 3^2 full factorial design for prepared effervescent tablets of ketorolac tromethamine

Ingredients (mg)	Batch code								
	OF-1	OF-2	OF-3	OF-4	OF-5	OF-6	OF-7	OF-8	OF-9
Ketorolac tromethamine	10	10	10	10	10	10	10	10	10
HPMCK4M	65 (−1)	75 (0)	85 (+1)	65 (−1)	75 (0)	85 (+1)	65 (−1)	75 (0)	85 (+1)
HPMCK15M	85	85	85	85	85	85	85	85	85
$NaHCO^3$	75 (−1)	75 (−1)	75 (−1)	80 (0)	80 (0)	80 (0)	85 (+1)	85 (+1)	85 (+1)
Ethylcellulose	55	45	35	50	40	30	45	35	25
Lactose	20	20	20	20	20	20	20	20	20
PVP: IPA (5%)	7	7	7	7	7	7	7	7	7
Magnesium stearate	15	15	15	15	15	15	15	15	15
Talc	3	3	3	3	3	3	3	3	3
Total (mg)	335	335	335	335	335	335	335	335	335

Independent variable level: Low (−1), Medium (0), High (+1). HPMC: Hydroxypropyl methylcellulose, $NaHCO_3$: Sodium bicarbonate, PVP: Polyvinylpyrrolidone, IPA: Isopropyl alcohol

Evaluation of HBS of ketorolac tromethamine

Weight variation, hardness, thickness, friability test, and drug content

According to the Indian Pharmacopoeia, all tablet characteristics were evaluated.[10] 20 tablets were individually weighed for the weight variation test, and the average weight (AW) was determined. The upper and lower limits for the weight variation test were established by applying the limit based on AW, followed by the calculated value (CV). The formula used to determine the upper and lower limits is AW+CV for the upper limit and AW-CV for the lower limit.[11] The "Monsanto" hardness tester was used to perform the hardness test, which involved fitting five randomly chosen tablets between the spindle and the anvil through their diameter. Next, moving the knurled knob raises the pressure applied to the tablet until the tablet breaks. The scale records the amount of force (in kg) needed to break the tablet.[12] The tablet was placed between two vernier caliper arms to measure the thickness. The thickness of each of the five tablets was measured.[13] The friability test was conducted to determine how friction and shock would impact the material. 20 tablets from the previously weighed sample were put in the Electrolab friability tester, which was rotated for about 4 min and a speed of 25 rpm was maintained. The tablets were cleaned and weighed again, and an equation was used to determine the percentage of friability. No more than 1% of the weight of compressed tablets should be lost.[14] The formula for determining friability is given in equation (1):

$$Friability = \frac{Initial\ weight - Final\ weight}{Initial\ weight} \times 100$$

The prepared HBS of ketorolac tromethamine was tested for its drug content. Five tablets were weighed and crushed to powder. A powder quantity equivalent to 100 mg of the drug was taken in a 100 mL volumetric flask. Then, it was dissolved in 5 mL methanol and made up the volume with 0.1 N HCl. The sample was mixed and filtered using Whatman filter paper, making suitable dilutions. Then, absorbance powder solution was measured at 313 nm using a Shimadzu UV 1800 spectrophotometer. The amount of drug was calculated using a standard calibration curve. ($n = 3 \pm SD$).[15]

In vitro buoyancy studies[16,17]

The buoyancy lag time was used to calculate *in vitro* buoyancy. The tablets were placed in a 250 mL beaker with 200 mL of 0.1N HCl. TFT (h) was calculated as the amount of time the tablet spent continuously floating on the medium's surface and the amount of time it required for the tablet to rise to the surface and float was determined as FLT (s).

SI[18]

After measuring the weight of the tablets (W_t), they were placed in the flask of dissolution apparatus USP- II (electrolab-08TDT) with 900 mL of 0.1N HCl (pH 1.2) to determine the SI. The weight of the tablet (W_0) was then calculated at different time intervals of 2, 4, 6, and 8 h after using blotting paper to remove excess fluid. Each experiment was performed in triplicates. The SI is measured in terms of % weight gain as given by the equation below,

$$SI = \frac{(Wt - Wo)}{Wo} \times 100$$

where W_t and W_0 are the final weight of the tablet at time t and the initial weight of the tablet, respectively.

In vitro drug release study

The drug release study was carried out using the USP type-II (rotating paddle) dissolution test apparatus (Electrolab 08TDT) containing 900 mL of 0.1 N HCl and rotated at a speed of 50 rpm with a temperature of 37 ± 0.5°C. As per the pharmacopeial method, 5 mL of the sample was withdrawn at pre-defined time intervals. After appropriate aliquots, the samples were analyzed for drug release estimation by detecting absorbance at 313 nm with a UV-visible spectrophotometer (Shimadzu UV1800). The samplings were carried out in triplicate ($n = 3$). To determine the drug release mechanism, the dissolution profiles of all formulations were submitted to kinetic modeling using zero-order, first-order, Higuchi, and Korsmeyer-Peppas models.[19] The data were analyzed using PCP Disso V3 software (Poona College of Pharmacy, IICP, Pune, India).

Characterization study by Fourier transform-infrared (FT-IR) spectroscopy

The FT-IR measurements of pure drug and HBS loaded with ketorolac tromethamine were obtained on FTIR spectrometer-430 (Jasco, Japan) using IR solution software. At room temperature, the spectra of all samples were scanned from 4500 to 350 cm^{-1}.[20]

Characterization study by differential scanning calorimetry (DSC) thermogram analysis

All formulated HBS batches were analyzed for DSC studies using DSC 60 (Shimadzu Corporation, Japan) instrument with indium as a reference standard. The prepared samples were heated throughout a temperature range of 25–400°C at a constant rate of 10°C/min while being thermally insulated in pans of perforated aluminum.[21]

Scanning electron microscopy (SEM)

The tablet's SEM picture was used to investigate the surface topography, texture, and morphology of the broken surface. SEM examination was performed on the optimized formulations (OF-7 and OF-8) using a JEOL SM6360A (Datum Ltd, Tokyo, Japan) scanning microscope.

Stability studies of selected formulations

To evaluate the accelerated stability, formulations of good drug release and buoyancy lag time were chosen for stability studies. These formulations were stored in a stability

chamber (Thermolab Scientific Equipments) for 3 months at a temperature of 40°C ± 2°C and 75% ± 5% RH, and various evaluation parameters including hardness, friability, FLT, drug content, and cumulative drug release were recorded. After the storage period, the formulations were tested in 0.1 N HCl (pH 1.2) for drug release.[22]

Student's t-test

The student's *t*-test was used to analyze the weight variation, hardness, thickness, friability test, drug content data, *in vitro* buoyancy, and stability studies. A value of $P < 0.05$ was considered significant.

Optimization of HBS of ketorolac tromethamine by response surface methodology (RSM)[23]

The HBS of ketorolac tromethamine was optimized using Design Expert Software (Design Expert version 11.0.3 State Inc., Minneapolis, MN). Based on pre-formulation study, a full 3^2 factorial design was constructed and conducted to optimize the levels of the independent variables such as HPMC K4M (X_1) and $NaHCO_3$ (X_2) which were studied at three levels each.

Throughout the trial, all other formulation and processing factors remained constant. Table 1 highlights the 9 experimental runs investigated, their factor combinations, and the conversion of the coded levels to the experimental units used during the study. The dependent variables selected for the study were hardness (Y_1), FLT (Y_2), TFT (Y_3), SI in 0.1N HCl (Y_4), and time taken to release 80% ($t_{80\%}$) of drug (Y_5). Statistical design is used to evaluate the effect of these independent variables on dependent variables or responses. The data analysis of parameters obtained from various batches for Y_1, Y_2, Y_3, Y_4, and Y_5 was subjected to multiple regression analysis. The term's positive or negative sign denotes the factor's positive (additive) or negative (antagonistic) impact on the reaction, respectively. This design was chosen because it gives enough degrees of freedom (d.f) to resolve both the major effects and the factor interactions.

RESULTS AND DISCUSSION

Weight variation, hardness, thickness, friability test, and drug content

The HBS of ketorolac tromethamine was off-white, smooth, and spherical shaped in appearance. The results of physicochemical characterizations are shown in Table 2. The weight variation is in the range of 330.75 ± 3.82–334.96 ± 3.75 mg ($P < 0.03$ $r^2 = 0.899$), hardness in the range of 2.83 ± 0.23–4.17 ± 0.07 kg/cm² ($P < 0.06$ $r^2 = 0.682$), and thickness in the range of 3.33 ± 0.04–3.99 ± 0.63 mm ($P < 0.001$, $r^2 = 0.992$), and the friability was found to be 0.295 ± 0.003–0.304 ± 0.001% ($P < 0.003$ $r^2 = 0.823$), respectively,

Table 2: Evaluation results of HBS of ketorolac tromethamine

Batches	Weight variation (mg) (*n*=20)		Hardness (kg/cm²) (*n*=5)		Thickness (mm) (*n*=5)		Friability (%) (*n*=20)		Drug content (%) (*n*=3)		Floating lag time (s) (*n*=5)		Total floating Time (h) (*n*=5)		Swelling index (%) (*n*=5)	
	Mean	SD(±)	Mean	SD(±)	Mean	SD(±)	Mean	SD(±)	Mean	SD(±)	Mean	SD(±)	Mean	SD(±)	Mean	SD(±)
OF1	333.65	2.29	2.83	0.23	3.99	0.63	0.29	0.01	98.40	0.58	109	0.05	24.3	0.01	48.20	0.35
OF2	330.75	3.82	3.16	0.11	3.58	0.42	0.30	0.01	99.47	0.45	85	0.02	24.22	0.02	49.00	0.56
OF3	333.34	4.37	3.33	0.07	3.53	0.41	0.30	0.02	97.09	1.35	116	0.06	24.08	0.05	49.30	0.23
OF4	334.69	3.26	3.68	0.04	3.33	0.04	0.30	0.02	98.55	0.86	59.08	0.02	24.11	0.02	47.80	0.89
OF5	332.30	4.96	3.81	0.03	3.37	0.07	0.30	0.03	99.28	0.42	58.22	0.03	24.04	0.02	50.03	0.45
OF6	331.60	4.19	3.96	0.02	3.35	0.06	0.30	0.01	99.40	0.24	51.71	0.03	24.00	0.01	50.30	0.25
OF7	332.77	4.01	4.00	0.02	3.57	0.48	0.29	0.05	98.63	1.77	38.25	0.09	24.55	0.01	48.00	0.21
OF8	333.19	4.26	4.14	0.03	3.41	0.05	0.29	0.02	98.82	0.82	37.36	0.05	24.46	0.03	52.30	0.46
OF9	334.96	3.75	4.17	0.07	3.40	0.03	0.29	0.01	101.98	2.26	35.24	0.04	24.10	0.01	54.33	0.11

where, OF-1 batch showed less friability of 0.295 ± 0.003 and OF-2 batch showed higher friability of 0.303 ± 0.001% and all the batches are within the limit of <1%. All the parameters were found to be significant ($P < 0.05$). The drug content values ranged from 97.09 ± 1.35 to 101.98 ± 2.26% ($P < 0.002$ $r^2 = 0.984$). These results give a reasonable hint that the formulations meet compendial requirements.[10] As a result, the formulations were studied further for performance criteria such as *in vitro* buoyancy, swelling, and percent drug release.

In vitro buoyancy studies

FLT was defined as the time delay with which the dosage form floats onto the GI fluid. The results are shown in Figure 1. Both the degree of polymer wetting and effervescence contribute to the floating of dosage form. The FLT of prepared batches was in the range of 35.24 ± 0.04–116 ± 0.06 s, while TFT of all batches showed buoyancy for 24 ± 0.01 h ($P < 0.0001$). The minimum FLT was shown by OF-9 (35.24 ± 0.04 s), followed by OF-8 and OF-7 (37.36 ± 0.05 and 38.25 ± 0.09 s), respectively. All the formulations produced effervescences required for floating and remained intact for 24 h. The above results showed the role of $NaHCO_3$ in enhancing the FLT. The batches OF-9, OF-8, and OF-7 content higher amount of $NaHCO_3$(85mg) as compared to other batches which lead to CO_2 generation in the presence of 0.1 N HCl and gas generated is trapped and protected within the gel, formed by hydration of HPMC K4M. This decreases the density of the tablet below 1, which makes the tablet to float in the GI content.[24]

SI

The swelling results in 0.1N HCl at 8 h were expressed in terms of SI as shown in Figure 2. It shows that as the polymer concentration increases, the water uptake increases. All the tablets made with HPMC K4 M and K15 M displayed good swelling both axially and radially. The swelling in pH 1.2 at 8 h ranged between 47.80 ± 0.89 and 54.33 ± 0.11 with significant difference ($P < 0.0001$). The rank order for swelling ratio was OF- 9>OF-8>OF-6>OF-5>OF-3>OF-2>OF-1>OF-7>OF-4 for pH 1.2 media. In general, the curves in 0.1 N HCl pH 1.2 exhibited an initial rapid increase in the first 30 min due to water entry through metastable pores and thereafter stayed constant. This is referred to as swelling hysteresis.[25] The OF-9 batch comprising of HPMC K4M and high concentration of $NaHCO_3$ had shown a gradual increase and thereafter constant swelling in 0.1 N HCl. To avoid the formation of an overly hydrated form that loses its integrity before drug release at the target, optimal swelling is required. The HBS's intact nature is necessary to sustain a delayed drug release throughout the GIT.[26]

In vitro drug release study

The drug release analysis was performed by taking data of 0.1N HCl pH 1.2 at 12 h as shown in Figure 3. More than 85% of drug are released at 12 h. The lowest drug release rate was obtained with OF-3, OF-6, and OF-9 batches which contain high amount of HPMC K4M (85 mg). The time taken to release 80% of the drug was taken into consideration and it was observed that the batchOF9 took

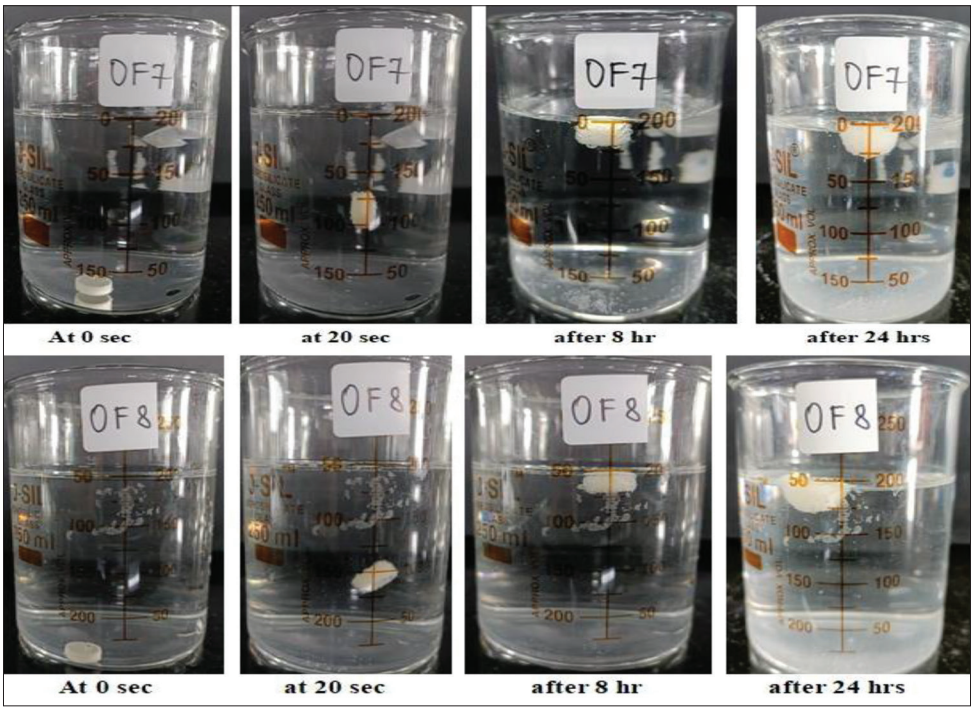

Figure 1: *In vitro* buoyancy studies of HBS of ketorolac tromethamine (OF-7 and OF-8)

around 11 h, followed by OF-6>OF-5>OF-3>OF-4>OF-2>OF-8>OF-1>OF-7. The batchOF7 releases 99.91% of the drug in 12 h which may be due to rapid swelling and bursting of the drug in dissolution media. As HPMC builds up, after its dissolution, an excessively viscous gel is formed around the tablets. This is more resistant to water penetration and erosion. Dissolved drug is released by diffusion through the viscous gel. Since the erosion rate of the swollen gel is slow compared with the rate of advance of the swelling front, the diffusion path length for the drug might increase with time, thus causing the release rate to decrease.[27] This may be because of HPMC which forms a hydrogel in contact with acidic fluid and helps the tablets to swell and float. The HPMC grade K4M is high viscosity grade of hydroxypropyl methylcellulose and forms a better matrix, in which CO_2 released from gas generating agent (sodium bicarbonate), than many of the other polymers. To study the exact mechanism of the drug release from floating tablets, drug release data were analyzed according to the zero order, first order, Korsmeyer-Peppas equation, Hixson–Crowell, and matrix model [Table 3]. The criterion for selecting the most appropriate model was chosen based on goodness of fit test. In case of OF-1, OF-3, OF-5, OF-6, and OF-9, the best fit model was matrix and for OF-2, OF-4, OF-7, and OF-8, it was first-order model. Using Korsmeyer-Peppas model, value of exponent *n* was calculated. The value of *n* for formulations OF-1 to OF-9 was in the range of 0.48–0.60 which indicates the drug transport mechanism to be anomalous transport (*n* = 0.45–0.89).[28]

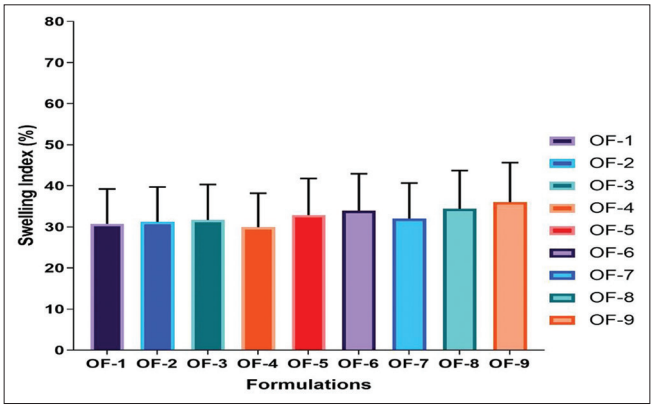

Figure 2: Swelling index at 8 h in 0.1N HCl (pH 1.2) of HBS of OF-1 to OF-9 (mean ± SD) (*n* = 3)

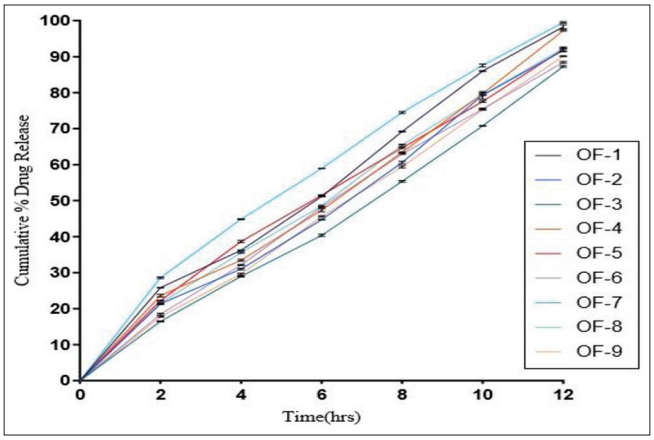

Figure 3: *In vitro* dissolution profile of HBS of ketorolac tromethamine. (OF-1 to OF-9) (mean ± SD) (*n* = 3)

Characterization study by FTIR spectroscopy

FTIR spectrophotometry was carried out for identification and affinity of formulation composition. The FT-IR spectra of ketorolac tromethamine show O-H stretching at 2916 cm^{-1}, N-H stretching at 3345 cm^{-1}, aromatic C-H stretching at 2848 cm^{-1}, C=O stretching at 1651 cm^{-1}, while C=C and C- N stretching show at 1579 and 1244 cm^{-1}, respectively. O-H N-H bending was shown at 1498 cm^{-1}. The FTIR study confirmed the identity of the drug and revealed the compatibility of the drug with other polymers. Likewise, the spectra of remaining formulations show resemblance with the spectra of drug indicating that the amalgam of drug and excipients did not show any major shifting or loss of functional peaks between them.[29]

Table 3: Model fitting of *in vitro* release data using correlation coefficient (r^2) and *n* value

Batch	Zero order	First order	Matrix (Higuchi)	Hixson Crowell	Korsmeyer Peppas		Best fit model	Drug release mechanism
	r^2	r^2	r^2	r^2	r^2	*n*		
OF-1	0.900	0.903	0.857	0.902	0.681	0.573	First order	Non-Fickian
OF-2	0.929	0.869	0.982	0.911	0.912	0.597	Matrix	Non-Fickian
OF-3	0.969	0.974	0.886	0.894	0.915	0.594	First order	Non-Fickian
OF-4	0.916	0.912	0.984	0.921	0.899	0.607	Matrix	Non-Fickian
OF-5	0.946	0.978	0.894	0.892	0.892	0.577	First order	Non-Fickian
OF-6	0.944	0.992	0.875	0.958	0.902	0.488	First order	Non-Fickian
OF-7	0.895	0.859	0.958	0.902	0.929	0.791	Matrix	Non-Fickian
OF-8	0.896	0.892	0.981	0.925	0.938	0.483	Matrix	Non-Fickian
OF-9	0.911	0.983	0.867	0.915	0.945	0.563	First order	Non-Fickian

Characterization study by DSC thermogram analysis

Figure 4 shows the DSC thermograms for all nine formulations (OF-1 to OF-9). When DSC studies were carried out under atmospheric condition, no significant change in thermal behavior was noted. All the nine formulated batches show DSC thermogram in the range of 161–163°C which indicates that there is no any significant shift in the position of ketorolac tromethamine melting endothermic peak. The results confirmed the absence of incompatibilities between drug and other excipients which are used in the formulation. The overlay thermogram of the OF-1 to OF-9 batches shows the presence of drugs, polymers, and additives in their initial forms and verifies that no chemical reaction(s) or physical state change occurs when these materials are mixed.[29]

SEM

Figure 5 shows SEM images of optimized batches (OF-7 and OF-8) before dissolution. Before disintegrating, the tablets had an unbroken surface with no perforations, channels, or troughs. After being exposed to the dissolving media, the drug diffuses out of the matrix. These SEM figures supported the theory of diffusion for the drug release mechanism from the developed gastroretentive drug delivery system.[30]

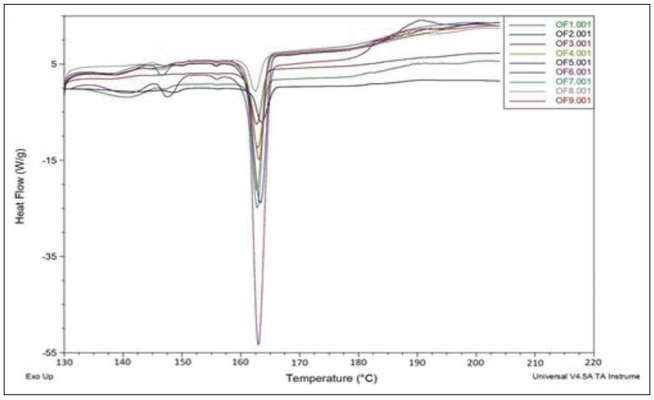

Figure 4: DSC thermograms of OF-1 to OF-9 batches

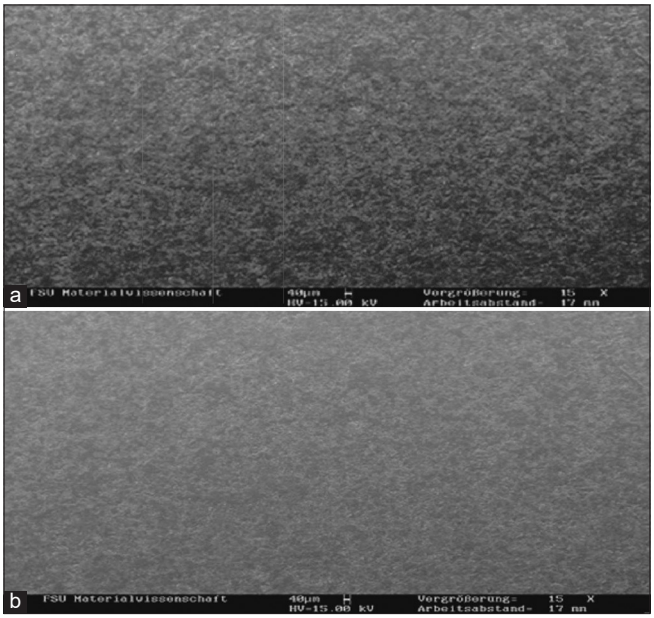

Figure 5: SEM images of HBS at 250 × (a) OF-7 (b) OF-8

Stability studies of selected formulations

Table 4 shows stability testing results after 3 months for batch OF-7 and OF-8. In view of their potential utility, stability studies for OF-7 and OF-8 were carried out at 40 ± 2°C and 75 ± 5% RH for 6 months (climatic zone IV conditions for accelerated testing) to assess their long-term (2 years) stability. The procedure for the stability studies followed the WHO document's suggestion for verifying the stability of items intended for the worldwide market. After storage, the formulations were examined for physical changes and tested for hardness, friability, drug assay, FLT, and *in vitro* drug release. There was no significant difference ($P < 0.05$) in the cumulative percent drug release of ketorolac tromethamine in 0.1N HCl of pH 1.2 for both OF-7 and OF-8 stored at 40 ± 2°C/75 ± 5% RH for 3 months and when compared to that released from the same formulations before storage. The insignificant change in the physical appearance, hardness, friability, drug assay, FLT, and *in vitro* drug release studies of OF-7 and OF-8 formulations after 3 months of storage at 40 ± 2°C/75± 5% RH indicates that the formulations could provide a minimum shelf life of 24 months.[31]

Statistical analysis

Statistical design is used to evaluate the effect of these independent variables on dependent variables or responses. The data analysis of parameters obtained from various batches for Y_1, Y_2, Y_3, Y_4, and Y_5 was subjected to multiple regression analysis.

Hardness

Based on the MLRA model, which is represented in the equation given below, the equation is plotted for hardness as follows.

$$Y_1=3.82+0.1583X_1+0.4983X_2-0.0825X_1X_2-0.0274X_1^2-0.1974X_2^2$$

(SD = 0.027; $r^2 = 0.997$)

Here, X_1 and X_2 represent the effect of variables that are concentration of HPMC K4M and $NaHCO_3$, respectively, and both variables show significant effect on hardness. The most significant effect on Y_1 was shown by the amount of HPMC K4M ($P < 0.0001$). This is obvious since the high hardness value could be observed only in tablets with large proportions of HPMCK4M (85 mg).[32] $NaHCO_3$ also shows that significant effect on hardness, the reason for this occurrence, is the smaller particle size of $NaHCO_3$. This could

Table 4: Stability data of OF-7 and OF-8 formulations before and after storage at 40°C/75% RH for 6 months

Evaluation parameter	OF-7 Batch					OF-8 Batch				
	Before storage	After storage	d.f	*P*-value	Significant or/not	Before storage	After storage	d.f	*P*-value	Significant or/not
Hardness (kg/cm^2)	4.00±0.02	3.93±0.09	2	0.04	Significant	4.14±0.03	3.92±0.03	2	0.05	Significant
Friability (%)	0.297±0.05	0.303±0.01	2	0.032	Significant	0.297±0.02	0.305±0.01	2	0.008	Significant
Drug content (90–110%)	98.63±1.77	97.45±0.45	2	0.05	Significant	98.82±0.82	97.90±0.45	2	0.03	Significant
Cumu. Drug release (%)	99.6±0.4	98.7±0.4	2	0.011	Significant	99.7±0.05	99.2±0.30	2	0.006	Significant
Floating lag time (s)	48.25	48.98	2	0.03	Significant	37.36	37.29	2	0.01	Significant

(*n*=3 with the mean±SD) (*P*<0.05); d.f: Degree of freedom

be explained by the fact that the tablet hardness decreased as the particle size of the compound increased.[33] This was correlated with the results obtained after the evaluation of formulated batches; in which OF-9 shows higher hardness due to the high concentration of HPMC K4M, meanwhile OF-1 batch containing low concentration shows lower hardness. In this case, the model term for hardness of the tablet was found to be significant ($P < 0.05$), as determined using ANOVA, as per the provision of Design Expert Software. The model F-value 427.68 implies that the model is significant which is shown in Table 5. Effects of independent variables on hardness (Y_1) are presented by Response Surface graph and 2-D contour plots as shown in Figure 6a and b, respectively. HPMC K4M had smaller particle size than other grades of HPMC and involved slightly narrower size distribution. However, the hardness contour plot showed a small blue area at the corner denoting HPMCK4M, referring to low hardness when the tablet formulation contained low concentration of HPMCK4M. Hence, the high hardness value may have been due to particle–particle interactions of suitable ratios of HPMCK4M and $NaHCO_3$.[34]

FLT

The final empirical model in terms of a coded factor for FLT (Y_2) is shown in Eq.

$$Y_2=63.29+0.5633X_1+33.19X_2$$

(SD = 10.46; $r^2 = 0.858$)

The equation represents the positive effects of HPMC K4M (X_1) and $NaHCO_3$(X_2) upon the FLT. The floating tablets were composed of $NaHCO_3$ as gas forming agent and combination of HPMC K4M as swelling matrix. Upon contact with the acidic medium (0.1NHCl), the fluid permeates into the matrix and initiates effervescence reaction. The liberated CO_2 is entrapped within the polymeric network. Consequently, polymer matrix swells rapidly and the swollen tablet achieves a required density which initiates it to float, reaches on the surface and remains buoyant for a long time as long as it maintains the required buoyancy.[35] The summary of ANOVA response is given in Table 6. In this case, the model term for FLT of the tablet was found to be significant ($P < 0.05$). Effects of independent variables on FLT (Y_2) are presented by Response Surface graph and 2-D contour plots as shown in Figure 7a and b, respectively. However, from contour plots, it is evident that $NaHCO_3$ has significantly influenced on FLT when its amount is 85 mg, which produced the minimum FLT for batches OF-3, OF-6, and OF-9. This value may be considered as a requirement for minimum effervescence to equilibrate gravitational force with buoyancy force exerted on the tablet while floating.

TFT

Based on the MLRA model, which is represented in the equation given below for TFT

Table 5: ANOVA for response surface quadratic model for Hardness. Analysis of variance table (partial sum of square - Type III)

Source	Sum of squares	d.f.	Mean square	F-value	*P*-value	
Model	1.810	5	0.361	472.68	<0.0001	Significant
X_1	0.150	1	0.150	196.48	<0.0001	
X_2	1.490	1	1.490	1946.31	<0.0001	
X_1X_2	0.027	1	0.027	35.56	0.0006	
$X_1$2	0.002	1	0.002	2.17	0.1436	
$X_2$2	0.107	1	0.107	140.60	<0.0001	
Residual	0.005	7	0.000			
Lack of fit	0.005	3	0.001			
Pure error	0.000	4	0.000			
Cor total	1.81	12				

X_1=HPMC K4M, X_2=$NaHCO_3$, DF: Degrees of freedom

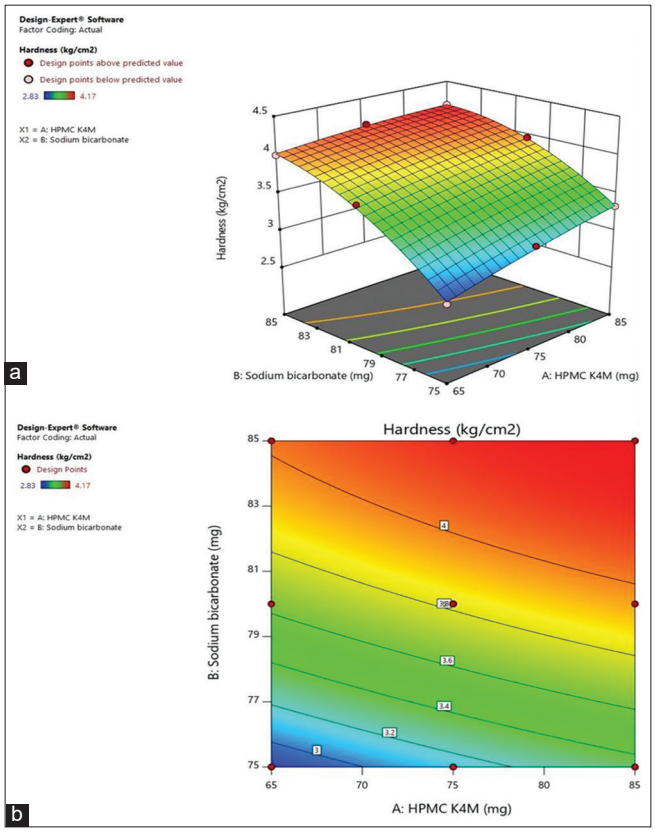

Figure 6: (a) Response surface graph showing the effect of HPMC K4M and $NaHCO_3$ on hardness of HBS of ketorolac tromethamine. (b) Contour plot showing the effect of HPMC K4M and $NaHCO_3$ on the hardness of HBS of ketorolac tromethamine

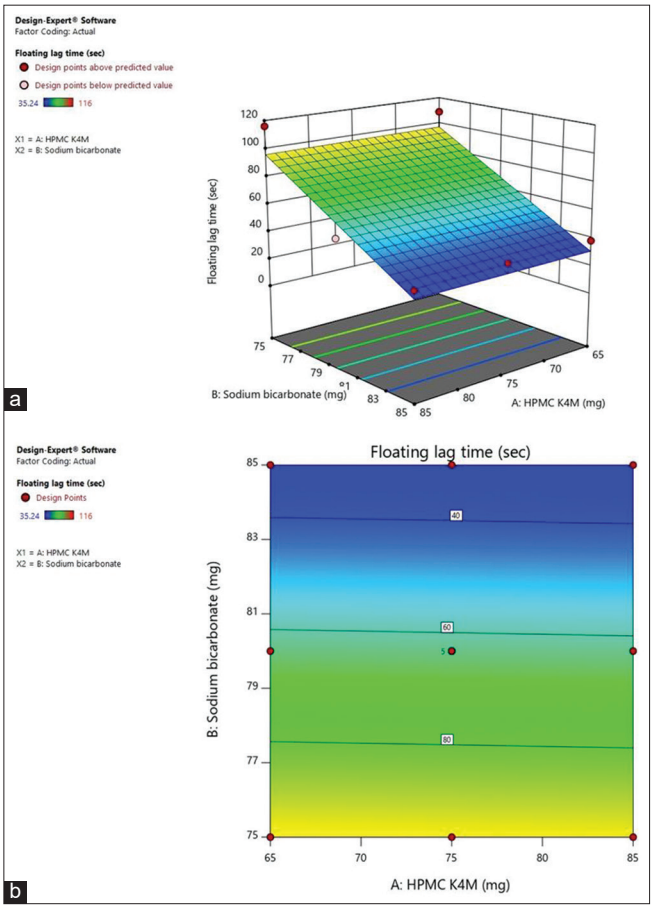

Figure 7: (a) Response surface graph showing the effect of HPMC K4M and $NaHCO_3$ on FLT of HBS of ketorolac tromethamine. (b) Contour plot showing the effect of HPMC K4M and $NaHCO_3$ on FLT of HBS of ketorolac tromethamine

$$Y_3 = 24.05 - 0.1300X_1 + 0.0850X_2 - 0.0575X_1X_2 - 0.0321X_1^2 + 0.2529X_2^2$$

(SD = 0.062; r^2 = 0.927)

By looking at the above equation, the HPMCK4M(X_1) shows negative effect, while $NaHCO_3$ (X_2) shows positive effect on TFT. Polynomial equation can be used to draw a conclusion after considering the magnitude of the coefficient and the mathematical sign it carries).[24] Due to high affinity of HPMCK4M toward water, which facilitates water penetration into tablet matrices and increases density, TFT

Table 6: ANOVA for response surface quadratic model for FLT. Analysis of variance table (partial sum of square - Type III)

Source	Sum of squares	d.f.	Mean square	F-value	*P*-value	
Model	6612.02	2	3306.01	30.23	<0.0001	Significant
X_1	1.90	1	1.90	0.0174	0.8976	
X_2	6610.12	1	6610.12	60.45	<0.0001	
Residual	1093.56	10	109.36			
Lack of fit	1093.56	6	182.26			
Pure error	0.0000	4	0.0000			
Cor total	7705.59	12				

X_1=HPMC K4M, X_2=$NaHCO_3$, d.f- degrees of freedom

gets increase as concentration of HPMCK4M increases. In addition, the HPMC K4M exhibits continual welling for a predetermined period of time. After that, it gets over hydrate and has no significant influence on TFT,[24] and hence, all the formulations show more than 24 h TFT. When concentration of $NaHCO_3$ increases, TFT increases which may be due to evolution and entrapment of CO_2 inside the hydrated polymeric matrices, resulting from the interaction between the gas generating agent ($NaHCO_3$) and dissolution medium (0.1NHCl) which leads to lowering of the density of matrices and increased the TFT.[36] In this case, the model term for TFT of the tablet was found to be significant ($P < 0.05$), as determined using ANOVA, as per the provision of Design Expert Software. The model F-value 17.97 implies that the model is significant as shown in Table 7. The correlation coefficient indicates a good fit. The response surface graph and contour plots for TFT are shown in Figure 8a and b, respectively.

SI

$$Y_4=58.61+1.65X_1+1.35X_2+1.30X_1X_2-6.02X_1^2-4.42X_2^2$$

The final empirical model in terms of a coded factor for SI in 0.1N HCl (Y_4) is shown in Eq.

(SD = 3.24; $r^2 = 0.791$)

The above equation states that the amount of HPMC K4M and $NaHCO_3$ shows positive effect on SI of prepared HBS of ketorolac tromethamine. However, it is observed that X_1 shows significant effect on SI as compared to X_2. The three-dimensional response surface graphs for SI are shown in Figure 9a and b, respectively. The results of ANOVA for the applied model on SI are given in Table 8. The significance of model was proved by the $P < 0.05$.[35] This gives the information about the main and interaction effects of the independent components.

Time taken for 80% of drug release ($t_{80\%}$)

$$Y_5=10.97+0.6667X_1-0.1667X_2-0.2500X_1X_2-0.3793X_1^2-0.8793X_2^2$$

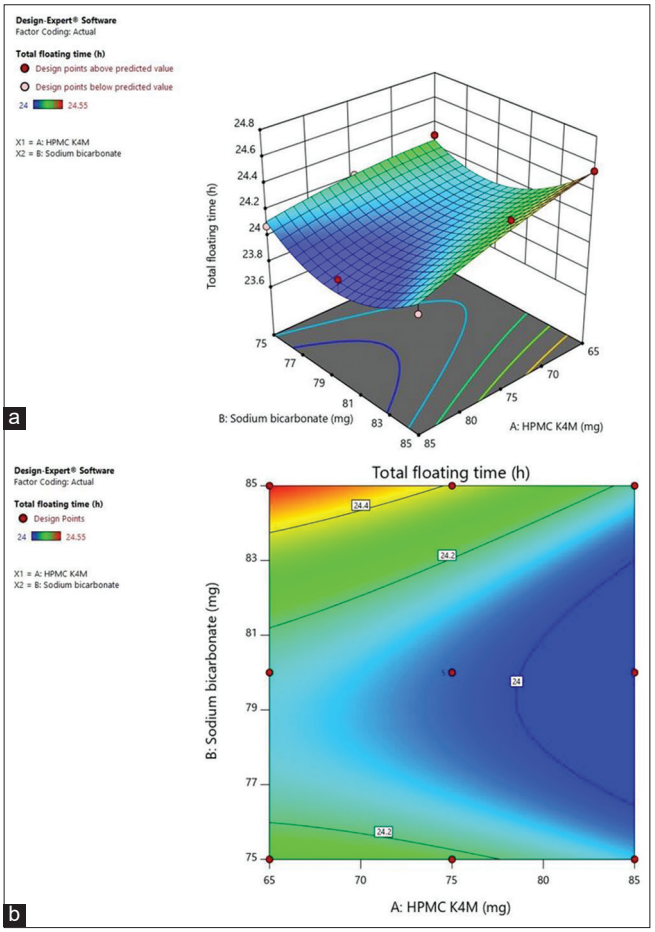

Figure 8: (a) Response surface graph showing the effect of HPMC K4M and $NaHCO_3$ on TFT of HBS of ketorolac tromethamine. (b) Contour plot showing the effect of HPMC K4M and $NaHCO_3$ on TFT of HBS of ketorolac tromethamine

The final empirical model in terms of a coded factor for $t_{80\%}$ (Y_5) is shown in Eq

(SD = 0.173; $r^2 = 0.970$)

In the above equation, X_1 and X_2 represent the effect of variables and it is observed that X_1 had significant effect on time taken for 80% of drug release ($t_{80\%}$). This means more

Table 7: ANOVA for response surface quadratic model for TFT. Analysis of variance table (partial sum of square - Type III)

Source	Sum of squares	d.f.	Mean square	F-value	*P*-value	
Model	0.348	5	0.069	17.970	0.0007	Significant
X_1	0.101	1	0.101	26.190	0.0014	
X_2	0.043	1	0.043	11.190	0.0123	
X_1X_2	0.013	1	0.013	3.420	0.1071	
$X_1$2	0.002	1	0.002	0.733	0.4201	
$X_2$2	0.176	1	0.176	45.630	0.0003	
Residual	0.027	7	0.003			
Lack of fit	0.027	3	0.009			
Pure error	0.000	4	0.000			
Cor total	0.375	12				

X_1=HPMC K4M, X_2=$NaHCO_3$, d.f- degrees of freedom

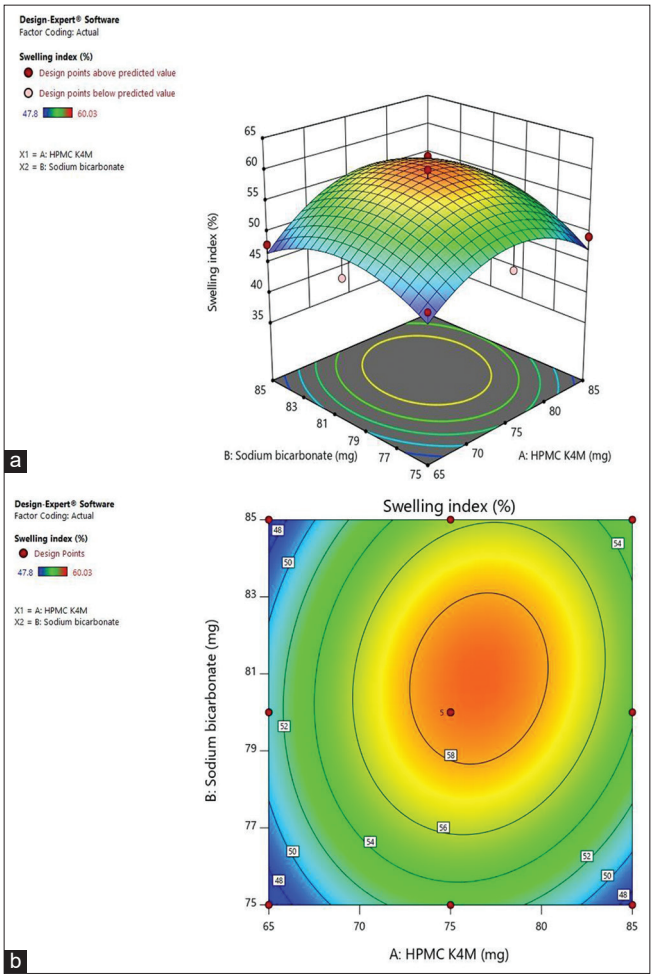

Figure 9: (a) Response surface graph showing the effect of HPMC K4M and sodium bicarbonate on swelling index (SI) of HBS of ketorolac tromethamine. (b) Contour plot showing the effect of HPMC K4M and sodium bicarbonate on SI of HBS of ketorolac tromethamine

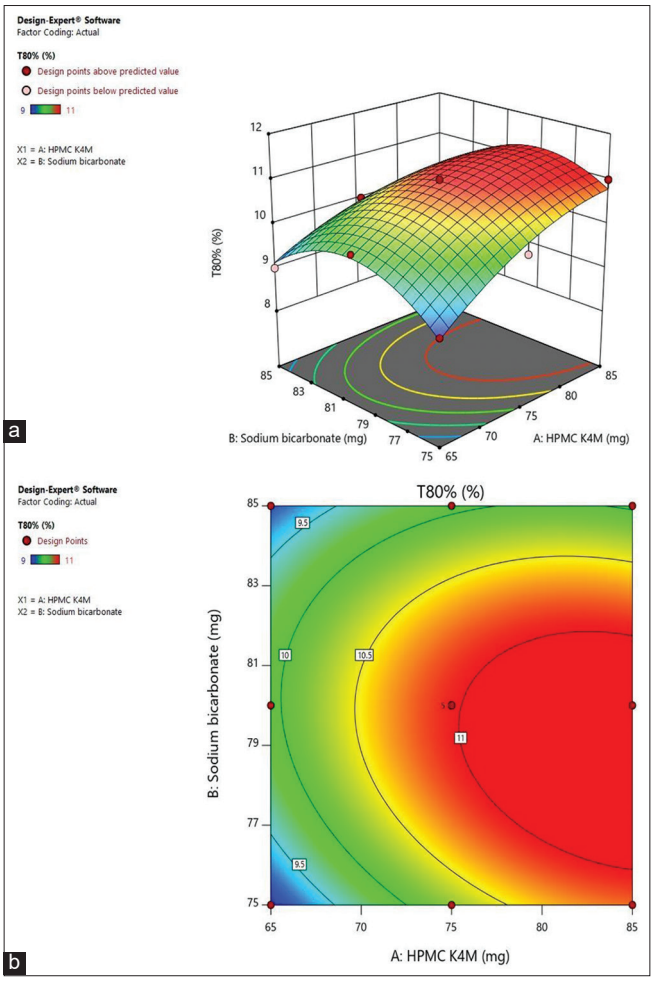

Figure 10: (a) Response surface graph showing the effect of HPMC K4M and $NaHCO_3$ on $t_{80\%}$ of HBS of ketorolac tromethamine. (b) Contour plot showing the effect of HPMC K4M and $NaHCO_3$ on $t_{80\%}$ of HBS of ketorolac tromethamine

the concentration of HPMC K4M, more time taken for 80% of drug release ($t_{80\%}$) is experienced by the formulation. The lowest drug release rate was obtained with OF-3, OF-6, and OF-9 batches which contains high amount of HPMC K4M (85 mg). Drug release was delayed in the formulations with increase in the concentration of HPMC K4M which was due to its higher hydrophilic ability. At higher polymer loading,

the viscosity of the gel matrix is increased which results in a decrease in the effective diffusion coefficient of the drug and hence decreased drug release into the dissolution medium. Furthermore, the gel layer formed was more viscous resulting in a greater retard in drug release.[36] The correlation coefficient indicates a good fit. In this case, the model term for time taken for 80% drug release of the tablet was found to be significant ($P < 0.05$) as determined using ANOVA, as per the provision of Design Expert Software.[37] The model F value 45.83 implies that the model is significant as given in Table 9. The response surface graphs for $t_{80\%}$ are shown in Figure 10a and b, respectively.

Validation of model

Responses were tested by additional random checkpoint batches covering the whole range of experimental domains to ensure the reliability of the evolved mathematical models.

Table 8: ANOVA for response surface quadratic model for swelling index. Analysis of variance table (partial sum of square - type III)

Source	Sum of squares	d.f.	Mean square	F-value	*P*-value	
Model	297.47	5	55.89	5.31	0.0247	Significant
X_1	16.33	1	16.33	1.55	0.2528	
X_2	10.93	1	10.93	1.04	0.3419	
X_1X_2	6.76	1	6.76	0.6427	0.4491	
X_12	100.00	1	100.00	9.51	0.0177	
X22	53.89	1	53.89	5.12	0.0580	
Residual	73.63	7	10.52			
Lack of fit	73.63	3	24.54			
Pure error	0.0000	4	0.0000			
Cor total	353.10	12				

X_1=HPMC K4M, X_2=$NaHCO_3$, d.f: Degrees of freedom

Table 9: ANOVA for response surface quadratic model for time taken to 80% drug release ($t_{80\%}$). Analysis of variance table (partial sum of square - type III)

Source	Sum of squares	d.f.	Mean square	F-value	*P*-value	
Model	6.87	5	1.37	45.83	<0.0001	Significant
X_1	2.67	1	2.67	88.99	<0.0001	
X_2	0.1667	1	0.1667	5.56	0.0505	
X_1X_2	0.2500	1	0.2500	8.34	0.0234	
X_1^2	0.3974	1	0.3974	13.26	0.0083	
X_2^2	2.14	1	2.14	71.26	<0.0001	
Residual	0.2098	7	0.0300			
Lack of fit	0.2098	3	0.0699			
Pure error	0.0000	4	0.0000			
Cor total	7.08	12				

X_1=HPMC K4M, X_2=$NaHCO_3$, d.f: Degrees of freedom

Table 10: Predicted and actual values of the responses for validation run

Responses	OF-7 batch		OF-8 batch	
	Predicted values	Actual values	Predicted values	Actual values
Hardness (kg/cm^2) (Y_1)	4.01	4	4.12	4.14
Floating lag time (s) (Y_2)	30.65	38.25	30.09	37.36
Total floating time (h) (Y_3)	24.54	26	24.39	24.1
Swelling index (%) (Y_4)	46.57	48	55.54	52.3
Time taken for 80% drug release ($t_{80\%}$) (Y_5)	9.12	9	9.91	10

Two batches were chosen using grid search analysis and the mathematical model predicted the replies. Two further batches were made and actual responses were recorded. Table 10 displays the observed and expected values from the experiment. According to the expected and experiential values of the responses, there was a close agreement of experimental values with predicted values for both the polymers HPMC K4M and $NaHCO_3$. This proved the predictability and validity of model and ascertained the effects of polymer; we may conclude that the model has good predictive ability.

CONCLUSION

The HBS tablets of ketorolac tromethamine could be prepared by wet granulation method using sodium bicarbonate (75–85 mg) and varying composition of HPMC K4M (65–85 mg). High floating ability of the formulation is likely to increase its GI residence time and eventually improves the extent of bioavailability. However, appropriate balancing between various levels of the polymers and floating agent is imperative to acquire proper controlled release and flotation of the formulation. High degree of prognosis is obtained using RSM which indicates that a 3^2 factorial design is quite efficient in optimizing drug delivery systems that exhibit non-linearity in response(s). From all the formulations, OB-8 showed ideal results in form of FLT, TFT, SI, and drug release ($t_{80\%}$), based on which it can be selected for *in vivo* study.

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