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Statistical optimization of floating-bioadhesive drug delivery system for risedronate sodium: *In vitro*, *ex vivo* and *in vivo* evaluation

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Abstract

The objective of the present investigation was to apply statistical design for the development of risedronate sodium floating-bioadhesive tablets (RSFBT) employing response surface methodology (RSM). A central composite design (CCD) was developed using Design of Expert (DOE) software to study the effect of formulation variables on the drug delivery system. The RSFBT were prepared by direct compression using hydroxy propyl methyl cellulose (HPMC K100M) as release retardant; carbopol (CP 974P) as bioadhesive polymer and sodium bicarbonate ($NaHCO_3$) as a gas-former. The quantities of HPMC K100M (X_1), CP 974P (X_2) and $NaHCO_3$ (X_3) were taken as independent variables and percentage drug release at 2 h (Q_2), 6 h (Q_6) and 12 h (Q_{12}), floating lag time (FLT), total floating time (TFT) and bioadhesive strength (BS) were selected as responses. The BS was determined using porcine gastric mucosa. In all 15 formulations were prepared and studied. The results of the CCD indicated that high levels of both X_1 and X_3, and low level of X_2 were required for the preparation of RSFBT. Further, a good correlation was observed between predicted and experimental values of the independent variables selected for this study. The drug release profiles of all the formulations were fitted into zero-order, first-order, Higuchi and Peppas models. The optimized formulation followed the Peppas model with a non-Fickian diffusion mechanism. The statistically optimized formulation (RSFBTsopt) was found to be physically stable when stored at 40 ± 2 °C/75 ± 5% RH for 3 months. *In vivo* evaluation of RSFBTsopt, $BaSO_4$-loaded tablets revealed a mean gastric retention time of 5 ± 0.86 h (n=3) in healthy volunteers.

Keywords: Floating-bioadhesive tablets, Central composite design, Design of Expert, I*n vitro* buoyancy, *Ex-vivo* bioadhesion, *In vivo* gastric residence time

Introduction

Sustained release (SR) formulations offer several pharmacokinetic and pharmacodynamic advantages over conventional dosage forms such as maintenance of constant therapeutic levels for prolonged period of time and minimize the fluctuations in plasma drug concentration. SR formulations might lower the risk of treatment failure [1], improve patient compliance by reducing dosing frequency and administration of total dose. However, SR dosage forms were not only developed to control the drug release for a specific period of time but also to prolong the residence time of the dosage form in the stomach or proximal part of small intestine. The presence of a dosage form in the upper part of gastrointestinal tract was important especially for drugs that are degraded or metabolised in the intestine or drugs had local action in the stomach [2, 3]. Further, for drugs with poor solubility in the intestine and those with site-specific absorption limitations, gastric retention approach might increase the overall gastrointestinal absorption [4]. Approaches to increase the gastric residence time of drug delivery systems include bioadhesive systems [5], swelling systems that increase their size [6, 7], low density systems [8], floating systems [9], high density systems [10], unfoldable and expandable systems, magnetic systems, and superporous, biodegradable hydrogel systems [11].

When the bulk density less than that of gastric fluids, floating drug delivery system (FDDS) remains buoyant in the stomach for a longer period of time without reducing the gastric emptying rate [12]. While the system floats on the gastric contents, the drug is released slowly at the desired rate from the system, resulting in an increased gastric retention time (GRT) and better control of fluctuations in plasma drug levels [13]. The main drawback of FDDS is that it is effective only when the fluid level in the stomach is sufficiently high. As the stomach empties and the tablet moves into the pylorus, which results the buoyancy of the dosage form may be retard [14]. This limitation can be overcome by using bioadhesive polymers to enable it to adhere to the mucous lining of

the stomach wall [15]. Floating- bioadhesive drug delivery systems offer the advantages of i) increased contact time with stomach mucosa, ii) more effective absorption and bioavailability of drugs with absorption window in the stomach and proximal intestine, and iii) reduced dosing frequencies [14].

Currently, statistical optimization is gaining importance in the formulation development. Response surface methodology (RSM) is an experimental design in which the factors involved and their relative importance can be assessed. RSM permits a deeper understanding of a process or product and has important applications like optimization and in establishing the robustness of the product. Central composite design (CCD) is a progression from the factorial designs which have been widely used in RSM and optimization [16].

Risedronate sodium (RS) is a potent pyridinyl bisphosphonate that binds to bone hydroxyapatite and inhibits osteoclast-mediated bone resorption. It is a third generation bisphosphonate. In preclinical studies risedronate demonstrated potent anti-osteoclast and anti-resorptive activity, increasing bone mass and biomechanical strength. RS is relatively rapidly absorbed from the upper gastrointestinal (GI) tract with a short biological half life of 1.5 h [17]. No gastroretentive floating-bioadhesive delivery system is reported for this drug till now. Due to these characters it is considered as a potential candidate for development of floating-bioadhesive drug delivery system.

The objective of the present investigation was to apply statistical design for the development of risedronate sodium floating-bioadhesive tablets (RSFBT) employing RSM. CCD was developed using Design of Expert (DOE) to study the effect of formulation variables on drug release, floating properties, swelling and *ex vivo* bioadhesion study. Physical stability of developed optimized formulation at 40 ± 2 ^{o}C/75 ± 5% RH for 3 months was studied. Further, the optimized formulation was investigated for *in vivo* radiological study to determine the gastric residence time in human volunteers.

Materials and Methods

Materials

Risedronate sodium (RS) was received as generous gift sample from M/s Hetero Drugs Ltd., Hyderabad, India. Hydroxypropyl methyl cellulose (HPMC K100M) was received as gift sample from M/s Orchid Pharma Ltd., Chennai, India. Carbopol (CP) 974P was received as gift sample from M/s Aurobindo Pharma. Ltd., Hyderabad, India. Sodium bicarbonate, microcrystalline cellulose (Avicel PH102), magnesium stearate (MS) and talc were purchased from S.D. Fine-Chem. Ltd., Mumbai, India. All other reagents used were of analytical grade.

Methods

Design of experiments

In this study, a CCD was used to optimize the formulation variables of RSFBT containing 3 factors and evaluated at 3 levels. The experimental trials were performed at all 15 possible combinations. The independent variables in our studies were: quantity of HPMC K100M (X_1), quantity of CP 974P (X_2) and quantity of sodium bicarbonate (X_3) and for each factor an experimental range was selected (Table 1) based on the results of preliminary experiments in our laboratory. The amount of MS and talc were kept constant at 1% w/w, while Avicel PH 102 was used as bulking agent to maintain constant tablet weight of 300 mg. The cumulative percentage drug released at 2 h (Y_1:Q_2), 6 h (Y_2:Q_6) and 12 h (Y_3:Q_{12}), floating lag time (FLT) in sec, (Y_4), total floating time (TFT) in hr, (Y_5) and bioadhesive strength (BS) in Newton, (Y_6) were included as responses. The experiments were designed by using DOE software (Version 8.0.7.1, Stat-Ease Inc., Minneapolis, MN, USA) and the layout of the design is shown in Table 2. The DOE software was used to give information not only on the critical values required to achieve the desired response but also the possible interactions of the selected independent variables on the dependent variables.

Table 1: Independent variables and their levels used in the central composite design.

Independent variables	Levels used, actual (coded)		
	Low (-1)	Medium (0)	High (+1)
X_1:quantity of HPMC K100M (mg)	40	80	120
X_2:quantity of CP 974P (mg)	20	40	60
X_3:quantity of $NaHCO_3$ (mg)	30	45	60

Table 2: Formulations with levels of independent variables and observed responses.

Formulation	Independent variables			Responses					
	X_1 (mg) [a]	X_2 (mg) [a]	X_3 (mg) [a]	Y_1 (%) [a]	Y_2 (%) [a]	Y_3 (%) [a]	Y_4 (sec) [a]	Y_5 (h) [a]	Y_6 (N) [b]
F1	40	20	30	31.38	50.98	74.53	66.67	12	0.352
F2	120	20	60	31.94	63.4	99.96	25.33	12	1
F3	80	40	19.77	24.79	38.57	52.29	NF	NF	0.841
F4	120	20	30	19.78	29.66	45.07	89.67	12	0.96
F5	40	60	30	23.9	36.09	57.17	73.83	5.1	0.625
F6	80	40	70.23	31.19	63.96	99.89	9.67	12	0.922
F7	12.73	40	45	78.53	101.41	101.21	21.5	2	0.35
F8	147.27	40	45	22.55	36.85	54.53	55.5	12	1.114
F9	40	60	60	55.83	98.91	100.91	21.83	7.2	0.6
F10	80	6.36	45	32.76	67.21	100.95	15.67	12	0.568
F11	80	73.64	45	25.85	37	48.2	55.67	8.3	1.138
F12	80	40	45	27.1	43.98	60.13	28.83	12	0.88
F13	40	20	60	36.78	74.45	100.84	26.67	12	0.358
F14	120	60	60	27.96	48.14	62.27	34.17	12	1.278
F15	120	60	30	16.91	24.74	36.43	139.17	8.2	1.217

Independent variables-X_1:HPMC K100M, X_2:CP 974P and X_3:$NaHCO_3$; responses-Y_1,Y_2 and Y_3 were cumulative percentage drug released at 2, 6 and 12 h respectively, Y_4:FLT, Y_5:TFT and Y_6:bioadhesive strength. NF: Not floated.
[a] Response values: average, n=6; [b] Response values: average, n=3.

Preparation of risedronate sodium floating-bioadhesive tablets

The experimental tablets were prepared at all possible combinations by direct compression. Required quantities of RS, HPMC K100M, CP 974P, $NaHCO_3$ and Avicel PH102 were weighed individually using electronic balance (AUX220, Shimadzu, Japan) and passed through sieve no. 40 to get uniform sized particles, then they were taken in a mortar and triturated for 10 min with the help of a pestle. Then the mixture was transferred into a polyethylene bag and further mixed for 5 min to ensure a homogeneous mass. The mixture was lubricated with 1% w/w MS and talc for additional 2 min and this lubricated blend was compressed into tablets using 8 mm flat- faced round punches on16 station punching machine (Riddhi, RDD3 Ahmedabad, India). The hardness of the tablets was adjusted to 6 kg/cm^2.

Determination of *in vitro* buoyancy

The *in vitro* buoyancy of RSFBT was determined in six replicates using United States Pharmacopoeia (USP) dissolution apparatus II (Electrolab, TDT-06T, Mumbai, India) in 900 ml of 0.1 N HCl maintained at 37 ± 0.5 C with paddle rotation of 50 rpm [18]. The FLT as well as TFT were determined visually. The time taken by the tablet to emerge onto surface of dissolution medium and the total time, the tablet remained buoyant on fluid surface were noted as FLT and TFT, respectively for all the formulations.

In vitro drug release studies

The *in vitro* dissolution of prepared RSFBT was studied in six replicates using USP dissolution apparatus II (Electrolab, TDT-06T, Mumbai, India). The dissolution medium was 900 ml of 0.1 N HCl (pH1.2), temperature was maintained at 37 ± 0.5 ^oC with a paddle rotation at 50 rpm. Five ml of aliquots were withdrawn at predetermined time intervals by means of a syringe and immediately replaced with 5ml of fresh dissolution medium each time. Samples were filtered using membrane filter (0.45 µm) and suitably diluted with dissolution medium wherever necessary and absorbance of the samples was measured at λ_{max} 261 nm by using double beam UV-Visible spectrophotometer (Elico, SL 210, India).

Drug release analysis and kinetics

The *in vitro* drug release profiles were subjected to different kinetic models to explain the release kinetics for RSFBT. In this study, the *in vitro* drug release profiles were subjected to zero-order [19], first-order [20], Higuchi [21] and Korsmeyer-Peppas kinetic models [22]. The goodness of fit was evaluated using the correlation coefficient values (R^2).

Zero-order: $F = K_0t$; where F is the fraction of drug released at time t, and K_0 is the zero-order release constant.
First-order: ln (1 F) = K_1t; where F represents the fraction of drug released at time t, and K_1 is the first-order release constant.
Higuchi model: $F = K_Ht^{1/2}$; where F represents the fraction of drug released at time t, and K_H is the Higuchi constant.
Korsmeyer–Peppas model: $F = K_Pt^n$; where F represents the fraction of drug released at time t, K_P is the rate constant and n is the release exponent, indicative of the drug release mechanism. Further, the Korsmeyer–Peppas model was employed in the analysis of *in vitro* drug release behavior of these formulations to distinguish between competing release mechanisms. If a value of n 0.5, indicates the Fickian release mechanism. The value of n between 0.5 and 1 is an indication of non-Fickian release mechanism (both diffusion controlled and swelling controlled). When, n 1, it is case-II transport and this involves polymer dissolution and polymeric chain enlargement or relaxation.

Swelling studies

The swelling behaviour of the tablets was determined in triplicate as per reported method [23]. Initial weight (W_0) of the tablets was noted individually and placed separately in a glass beaker containing 200 ml of 0.1 N HCl, maintained at 37 ± 0.5 ºC. At regular time intervals, the tablets were removed and the excess surface liquid was carefully removed by means of a filter paper. The swollen matrix was then reweighed (W_1). The percentage swelling was calculated by using following equation.

$$\text{Percentage swelling} = \frac{(W1-W0)}{W0} \times 100$$

Ex vivo bioadhesion study

The bioadhesive strength of the RSFBT was determined using an ultra test (Mecmesin, West Sussex, UK) equipped with a 5-kg load cell. The porcine gastric mucosa was obtained from slaughterhouse and was stored in phosphate buffer solution prior to the bioadhesion study. The mucosal membrane was excised by removing the underlying connective tissue and was secured tightly to a circular stainless steel adapter of a diameter 2.2 cm provided with the equipment. This was fixed to advanced force gauze. The RSFBT to be tested was placed over another cylindrical stainless steel adaptor of similar diameter and mounted on the platform of motorized test stand. The tablet with a backing membrane was adhered on to it using a solution of cyanoacrylate adhesive. All measurements were conducted at room temperature. During the study, 100 µl of 1% w/v mucin solution was used to moisten the porcine gastric mucosal membrane [24]. The upper support was lowered at a speed of 0.5 mm/s until contact was made with the tissue at the predetermined force of 0.5 N for a contact time of 180 sec. At the end of the contact time upper support was withdrawn at a speed of 0.5 mm/s to detach the membrane from the tablet. Data collection and calculations were performed using the data plot software package of the instrument. The peak detachment force (maximum force required in Newton to detach the tablet from the mucosa) or bioadhesive strength (BS) was expressed as mean ± SD in triplicate of all formulations (Table 2).

Statistical analysis of the data and validation of the model

In this study, evaluation of the quality of fit of the model was performed employing DOE software. Polynomial models including linear, interaction and quadratic terms were generated for all the response variables using multiple linear regression analysis. The best fit model was selected based on comparison of several statistical parameters, including the coefficient of determination (R^2), adjusted R^2 and coefficient of variation (CV) provided by DOE software. Further, analysis of variance (ANOVA) was used to identify significant effects of factors on response regression coefficients. The F test and P values were also calculated using the software. The relationship between the dependent and independent variables was elucidated using response surface plots (Contour and 3-D surface). These plots were used to study the effect of various factors on the response at a given time and to predict the responses of dependent variables at intermediate levels of independent variables. Finally, a numerical optimization technique (desirability approach) and a graphical optimization technique (overlay plots) were used to generate new formulation with desired responses. To validate the chosen experimental design, the responses of experimental values were quantitatively compared with responses of predicted values and percentage relative error was calculated.

Physical stability studies

Physical stability studies were conducted according to International Conference on Harmonization (ICH) guidelines. The statistically optimized formulation (RSFBTsopt) was enclosed in polyethylene bottle and placed in a desiccator containing saturated sodium chloride solution, which provided 75 ± 5% RH. The desiccator was stored at 40 ± 2 ºC for 3 months [25]. At predetermined time intervals, the tablets were examined for hardness, drug content, in vitro buoyancy and drug release. Finally, the tablets were tested for any statistical difference using the Students paired t-test, the differences were considered to be significant at $p < 0.05$.

In vivo buoyancy study

All the ingredients used in this were transparent to x-ray, and therefore, to make the optimized tablet formulation (RSFBTsopt) x-ray opaque, 30 mg of the drug and 12 mg of Avicel PH 102 were replaced with barium sulphate ($BaSO_4$) and weight of all other ingredients were kept constant so that the final tablet weight remained same. This amount was determined experimentally to allow x-ray visibility but not to hinder tablet buoyancy. For *in vivo* radiological study, tablets with following composition were prepared: 1.66 % drug, 14 % $BaSO_4$, 39.13 % HPMC K100M, 6.66% carbopol 974P, 20 % $NaHCO_3$, 16.53 % Avicel PH 102, 1% magnesium stearate and 1 % talc. The tablets were prepared by direct compression method as described previously.

The *in vivo* gastric residence time of GRDDS can be determined by a variety of techniques such as x-ray, endoscopy, ϒ-scintigraphy [26]. In this work, x-ray technique was used to determine the gastric residence time of RSFBT. The protocol of radiological study was approved by the Institutional Ethics Committee, University College of Pharmaceutical Sciences, Kakatiya University, India. Three healthy male volunteers participated after giving an informed written consent. The subjects weighed in between 65–68 kg (66.3 ± 1.5 kg), in height from 165-167 cm (166 ± 1.0 cm) and in the age group of 25-34 years (29 ± 4.5 years). The study was conducted under the guidance of an expert radiologist. About 30 min before starting the study, the volunteers were fed with low calorie food having 100 g bread and 200 ml water. The optimized, $BaSO_4$-loaded RSFBTsopt was administered orally to every volunteer with 200 ml of water. During the study, the subjects were instructed to sit, and not allowed to move around and eat. At different time intervals like, 1, 2.5, 4.5 and 5.5 h, the volunteers were exposed to abdominal x-ray imaging in a standing position. The distance between source of x-rays and the subject was kept constant for all images. Thus, the observation of the tablet movements could be easily noticed [27].

Results and Discussion

The tablets were prepared by direct compression and evaluated for their physical characters. The tablet weights ranged between 298.50 ± 7.11 and 303.0 ± 6.75 mg, the hardness varied between 5.84 ± 0.43 and 6.08 ± 0.34 kg, thickness between 5.56 ± 0.094 and 5.62 ± 0.057 mm and friability ranged between 0.29 and 0.42 %. The drug content of all the formulations varied between 98.18 ± 2.01 and 101.20 ± 1.24 %. Thus, the physical parameters of the prepared tablets were within pharmacopoeial limits.

Determination of *in vitro* buoyancy

FLT (Y_4) of all formulations was within the range of 9.67 sec lowest is (F6) to 139.17 sec highest is (F15) and the results are shown in Table 2. All formulations floated (Y_5) in dissolution medium for more than 8 h (except F3, F5, F7 and F9), which indicated that good matrix integrity during the extended period of time. The results showed that as the concentration of X_1 increased from 12.73 mg in F7 to 147.27 mg/tablet in F8, the FLT (Y_4) decreased from 55.50 sec in F8 to 21.50 sec in F7 due to the increased hydrophilic nature of the polymer allowing penetration of water. And the TFT (Y_5) increased due to swelling of the tablet, which kept it intact for a longer period of time [28]. The concentration of X_2 also significantly influenced on Y_4 and Y_5 responses. As the concentration of X_2 increased from 6.36 mg in F10 to 73.40 mg/tablet in F11, the Y_4 was increased from 15.67 sec in F10 to 55.67 sec in F11 and Y_5 was decreased. Further, the X_3 also affected Y_4 and Y_5 responses. As the X_3 concentration increased from 19.77 mg in F3 to 70.23 mg/tablet in F6, the Y_4 was decreased from 28.83 sec in F12 to 9.67 sec in F6. Apart from this, the formulation F3 could not float on dissolution medium due to insufficient gas generated, to keep the tablets floating. In this work, $NaHCO_3$ was used as a gas-generating agent in order to aid floating of tablets. The $NaHCO_3$ induced CO_2 generation in the presence of dissolution medium (0.1 N HCl, pH 1.2). The gas generated was trapped and protected within the gel formed by hydration of the polymer, thus decreasing the density of the tablet below 1 gm/ml, leading to tablet buoyancy [29].

In vitro drug release studies

In vitro drug release of all fifteen formulations of RSFBT was carried out in 0.1 N HCl (pH 1.2). The study was performed for 12 h and drug release profiles are shown in Figure 1.

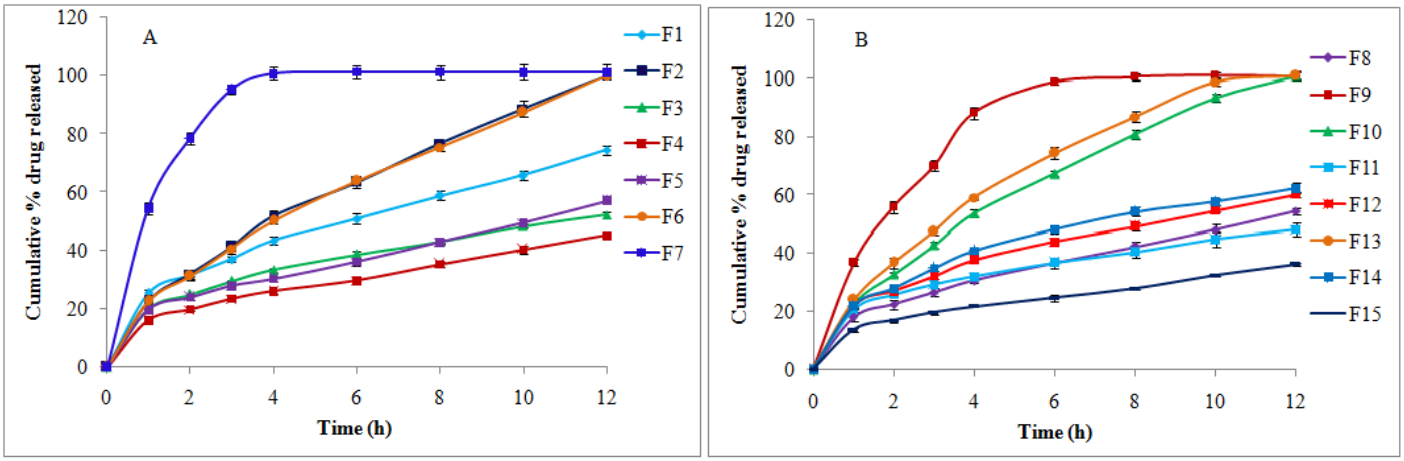

Figure 1: *In vitro* dissolution profiles of GFBT of RS A) formulations, F1 to F7 and B) formulations, F8 to F15 (n=6). SD bars are not visible.

Effect of concentration of HPMC K100M

The effect of concentration of X_1, 12.73 mg, 80 mg and 147.27 mg/tablet on drug release was evaluated and is shown in Figure 2. The maximum cumulative release of RS from the formulation F7

containing 12.73 mg/tablet was 100.81 ± 2.19 % at end of 4 h. Similarly, drug release from formulations F8 and F12 containing 147.27 mg and 80 mg/tablet was 54.52 ± 1.19 % and 60.13 ± 0.91 % respectively at 12 h (Y_3). The difference in drug release might be due to the amount of gel layer formed around the tablets. At higher concentration of X_1 resulted in a greater amount of gel being formed. This gel increased diffusion length so that drug release was decreased. When concentration of X_1 was 12.73 mg/tablet, the RSFBT could not retain its physical integrity for desired period of 12 h. If the physical integrity is not maintained, the tablet would break down into smaller fragments and escape from the upper part of the GIT [30]. As the concentration of X_1 was increased, the tablets could retain their physical integrity and the drug release was significantly extended.

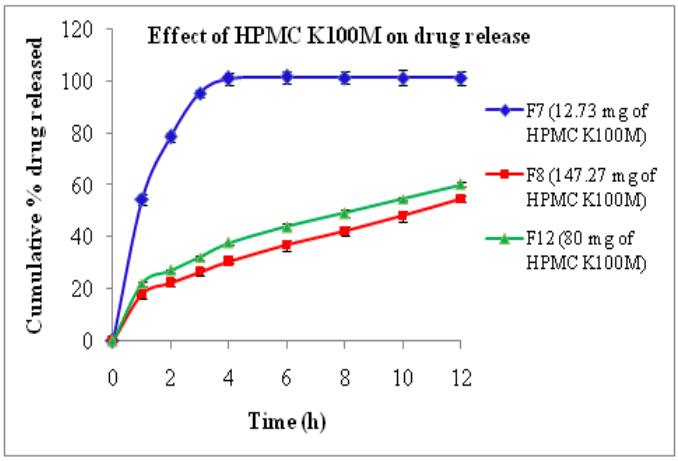

Figure 2: Effect of concentration of HPMC K100M on release of RS from GFBT (n=6). SD bars are not visible.

Effect of concentration of CP 974P

Carbomer 974P readily absorbs water and swells. In addition to its hydrophilic nature, cross-linked structure and insolubility in water makes the Carbopol 974P as a potential candidate for use in controlled release drug delivery systems.

The effect of CP 974P (X_2) concentration (6.36 mg, 73.64 mg and 40 mg/tablet) on the release was evaluated (Figure 3). The amount of drug released from the formulation F10 containing 6.36 mg/tablet was 100.94 ± 1.65 % at the end of 12 h (Y_3). Similarly, 48.19 ± 2.36 % and 60.13 ± 0.91 % of drug was released at the end of 12 h from formulations F11 and F12 containing 73.64 mg and 40 mg/tablet, respectively. Increase in concentration of X_2, decreased the drug release, which could be due to increased imbibition of water into the polymer. Further, Carbopol 974P (X_2) and HPMC K100M (X_1) produce a synergistic increase in viscosity due to the stronger hydrogen bonding between these two polymers [31]. This in turn formed stronger bridges between the two polymers resulting in a more rigid structure, which influenced drug diffusion to slow down.

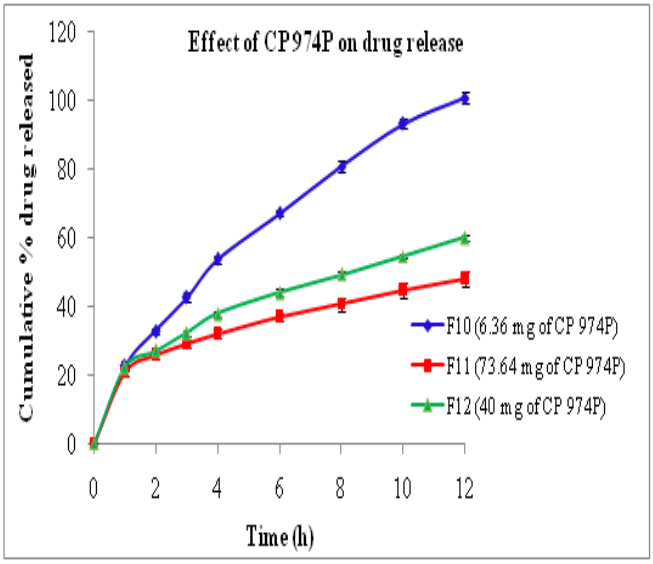

Figure 3: Effect of concentration of CP 974P on release of RS from GFBT (n=6). SD bars are not visible.

Effect of concentration of $NaHCO_3$

The RSFBT employed $NaHCO_3$ (X_3) as gas-forming agent to improve *in vitro* buoyancy. The influence of X_3 on cumulative drug release was also investigated (Figure 4). The cumulative amount of drug released from formulation F3 containing 19.77 mg/tablet was found to be 52.28 ± 1.19 % in 12 h (Y_4). Whereas, the amount of drug released from formulations F6 and F12 containing 70.23 mg and 45 mg/tablet was 99.89 ± 1.10 % and 60.13 ± 0.91 % respectively in 12 h. As the concentration of X_3 increased from 19.77 to 70.23 mg/tablet, drug release was significantly increased.

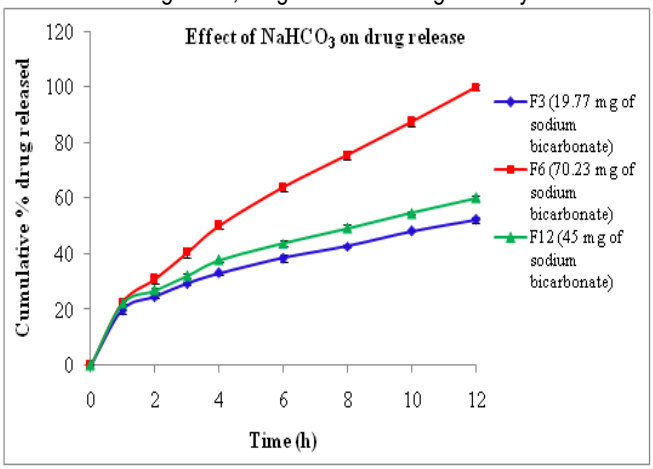

Figure 4: Effect of concentration of $NaHCO_3$ on release of RS from GFBT (n=6). SD bars are not visible.

Drug release analysis and kinetics

The in vitro drug release profiles of all the RSFBT formulations were subjected to different kinetic models. The n value with corresponding correlation coefficients (R^2) for all the formulations are shown in Table 3. Release of the drug from formulations F1,

F4, F5, F8, F9 and F15 followed Higuchi model due to high R^2 value (0.909 in F9 to 0.996 in F1) whereas that from F2, F3, F6 and F10-F14 followed Peppas model and the R^2 value ranged from 0.994 in F12 and F13 to 0.998 in F10 and F11. The value of release exponent n for all the RSFBT formulations ranged from 0.223 in F7 to 0.620 in F10. Release of the drug from formulations F2, F6, F10 and F13 involved non-Fickian diffusion (n values ranged from 0.596 in F13 to 0.620 in F10) indicated diffusion controlled and swelling controlled while those others involved Fickian diffusion indicated diffusion controlled. The release rate constants (k) of all the RSFBT formulations were significantly different. The k_p value for all the formulations was ranged from 13.15 in F15 to 64.71 in F7. Higher the value of k, greater the drug released

Table 3: Mathematical models and release kinetics of RS from FBT.

Formulation	Zero order		First order		Higuchi		Korsmeyer-Peppas		
	R^2	k_0	R^2	k_1	R^2	K_H	R^2	K_P	n
F1	0.903	5.20	0.978	0.100	0.996	98.21	0.990	24.37	0.437
F2	0.961	7.65	0.657	0.467	0.989	105.60	0.997	21.82	0.605
F3	0.858	3.58	0.931	0.053	0.990	96.83	0.997	19.36	0.391
F4	0.895	3.09	0.944	0.043	0.991	98.43	0.984	15.10	0.413
F5	0.913	3.92	0.962	0.060	0.986	98.83	0.969	18.07	0.424
F6	0.964	7.62	0.683	0.418	0.988	105.90	0.997	21.42	0.609
F7	0.477	5.71	0.680	0.371	0.755	72.71	0.745	64.71	0.223
F8	0.911	3.82	0.965	0.057	0.995	99.03	0.989	17.06	0.444

Swelling studies

The ability of hydration of the formulation is important because it effects on: (a) tablet buoyancy, (ii) adhesion ability of swellable polymers and (iii) drug release kinetics.

The maximum percentage of swelling was observed at the end of 12 h for all the formulations (Figure 5). There was a significant ($P < 0.05$) increase in swelling of the tablet containing varying amount of X_1 (F7, F8 and F12) with an increase in the X_1 content. Further, the amount of X_2 also influenced on tablet swelling. The tablet swelling was increased with an increase in X_2 content (F10, F11 and F12). In all the formulations, maximum swelling (344.91±6.35 %) was observed for formulation F8 containing 147.27 mg of X_1, 40 mg of X_2 and 45 mg of X_3.

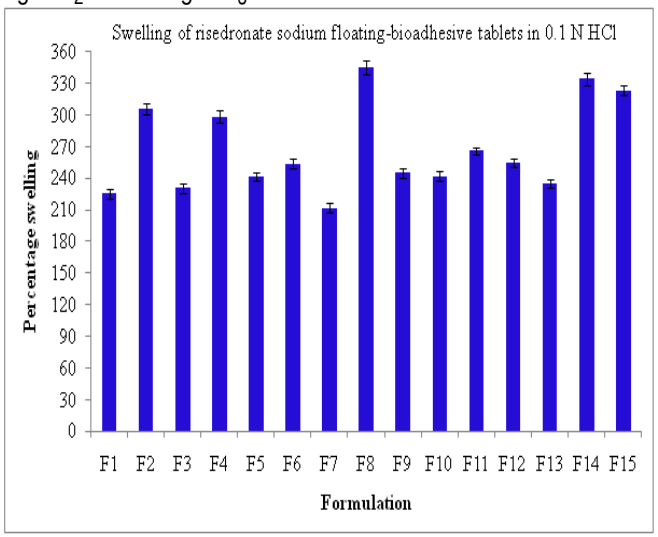

Figure 5: Effect of concentration of various excipients on swelling of GFBT of RS (n=3).

Ex-vivo bioadhesion study

Apart from *in vitro* buoyancy of the tablet, bioadhesive property could be an important property for gastroretentive drug delivery systems. The developed formulations contained CP 974P, which has bioadhesive property. HPMC polymers are also reported to have the bioadhesive property. Other materials used in the study such as $NaHCO_3$, Avicel pH 102, magnesium stearate and talc do not possess bioadhesive properties. The bioadhesive strength (BS) of the developed RSFBT is shown in Table 2. The effects of X_1 and X_2 and their interaction on Y_6 at a fixed level of X_3 are shown in Figure 7. At low level of X_2, Y_6 was found to be increased from 0.352 N in F1 to 1.0 N in F2 when the concentration of X_1 increased from 40 mg to 120 mg. Similarly, at high levels of X_2, the response Y_6 increased from 0.6 N in F9 to 1.278 N in F14 when X_1 increased from 40 mg to 120 mg. Whereas $NaHCO_3$ (X_3) could not show any effect on bioadhesion. The minimum Y_6 (0.350±0.026 N) was observed for formulation F7 containing 12.73 mg of X_1 and 40 mg of X_2. Similarly, formulation F14 showed maximum Y_6 (1.278±0.046 N), which contained 120 mg of X_1 and 60 mg of X_2. From the results, BS was found to be increased by increasing either of the components, HPMC K100M and CP 974P.

Statistical analysis of the data and validation of the model

The responses of all the formulations were fitted to linear, interaction or quadratic models using DOE software. A linear model is suggested for Q_2, Q_6, Q_{12} and FLT, interaction model for TFT, and quadratic model for BS. The calculated R^2 (Table 4) values for all the responses ranged from 0.7728 to 0.9991 indicating a good model. The adjusted and predicted R^2 values were 0.7047 and 0.5215 for Q_2, 0.7958 and 0.6759 for Q_6, 0.8550 and 0.7859 for Q_{12}, 0.7745 and 0.6548 for FLT, 0.8231 and 0.6029 for TFT and

0.9971 and 0.9898 for BS respectively. In all the responses, the adjusted and predicted R^2 values are in reasonable agreement. In all the cases, precision values ranged in between 9.8900 and 67.1819 indicating an adequate signal and the model can be navigated within the design space.

Table 4: Statistical parameters for the responses of RSFBT.

Parameter	Q_2 (%)	Q_6 (%)	Q_{12} (%)	FLT (%)	TFT (%)	Bioadhesive strength (N)
Mean	0.1907	0.1475	70.9407	1.5862	10.5231	-0.2401
SD	0.0145	0.0127	9.3055	0.1553	1.0188	0.0228
CV %	7.6131	8.5866	13.1173	9.7932	9.6814	9.5092
R^2	0.7728	0.8429	0.8884	0.8309	0.9116	0.9991
Adj R^2	0.7047	0.7958	0.8550	0.7745	0.8231	0.9971
Pred R^2	0.5215	0.6759	0.7859	0.6548	0.6029	0.9898
Adeq Precis	9.8900	12.7611	15.7206	10.9105	10.1446	67.1819

The application of RSM yielded the following regression equations.

$$Y_1(Q_2)=0.19+0.00044X_1+0.00023X_2–0.0011X_3 \quad (1)$$

$$Y_2(Q_6) = 0.16 + 0.00038X_1 + 0.00043X_2 – 0.0013X_3 \quad (2)$$

$$Y_3 (Q_{12}) = 65.94 – 0.28X_1 – 0.56X_2 + 1.12X_3 \quad (3)$$

$$Y_4 (FLT) = 2.16+0.0018X_1 + 0.0044X_2 – 0.019X_3 \quad (4)$$

$$Y_5 (TFT) = 18.69 – 0.040X_1 – 0.29X_2 – 0.072X_3 + 0.0012X_1X_2 + 0.00035X_1X_3 + 0.0025X_2X_3 \quad (5)$$

$$Y_6 (\text{Bioadhesive strength}) = -2.50 + 0.030X_1 + 0.02X_2 – 0.00019X_3 – 0.000095X_1X_2 + 0.000024X_1X_3 – 0.000021X_2X_3 – 0.00011X_1^2 – 0.000079X_2^2 + 0.0000023X_3^2 \quad (6)$$

The two-dimensional contour plots and three-dimensional response surface plots are shown in Figures 6 and 7. These plots are very useful to study the interaction effects of the factors on the responses, i.e., two factors on the response at one time. In all the presented figures, the third factor was kept at a constant level.

All the responses were optimized with different targets by a multicriteria decision approach (a numerical optimization technique by the desirability function and a graphical optimization technique by the overlay plot) and are shown in Figures 8 and 9. The optimized formulation was obtained by applying constraints on both dependent and independent variables. The constraints were: Q_2 26 ± 5 %, Q_6 56 ± 5%, Q_{12} 95 ± 5 % (fixed based on theoretical drug release profile calculation); minimal FLT; maximal TFT and maximal BS. These constrains remained same for all the formulations. The recommended constraints of the independent variables were calculated by the DOE software. From the above plot, the desirability was found to be 0.810. The extensive grid and feasibility searches provided the selection of optimum formulation by using desired response plot and over lay plot respectively.

The optimum values of selected independent variables obtained by using DOE software were 117.40 mg HPMC K100M (X_1), 20 mg CP 974P (X_2) and 60 mg $NaHCO_3$ (X_3). The final optimal tablet composition contained 117.40 mg HPMC K100M, 20 mg CP 974P, 60 mg $NaHCO_3$, 61.60 mg Avicel PH 102, 3 mg magnesium stearate and 3 mg talc. The statistically optimized formulation (RSFBTsopt) fulfilled all the physicochemical characters and had Q_2 30.72 %; Q_6 61.91 %; Q_{12} 97.25 %; FLT 20.83 s; TFT >12 h and BS 0.996 N. Release of the drug from RSFBTsopt followed Peppas model (R^2 = 0.996) with non-Fickian diffusion mechanism (n = 0.607 and K_p = 21.08). The observed responses of RSFBTsopt were in close agreement with the model predictions and the relative errors (%) was calculated (Table 5). The experimental values were in agreement with the predicted values confirming the predictability and validity of the model.

Physical stability studies

The RSFBTsopt was selected for stability studies. Before and after conducting the stability studies for 3 months, the results were analysed by using Student's paired t-test. No significant difference ($p > 0.05$) was observed in the tablet hardness, drug content, *in vitro* buoyancy or *in vitro* dissolution (Table 6). Therefore, the RSFBTsopt was found to be stable for at least 3 months under these storage conditions.

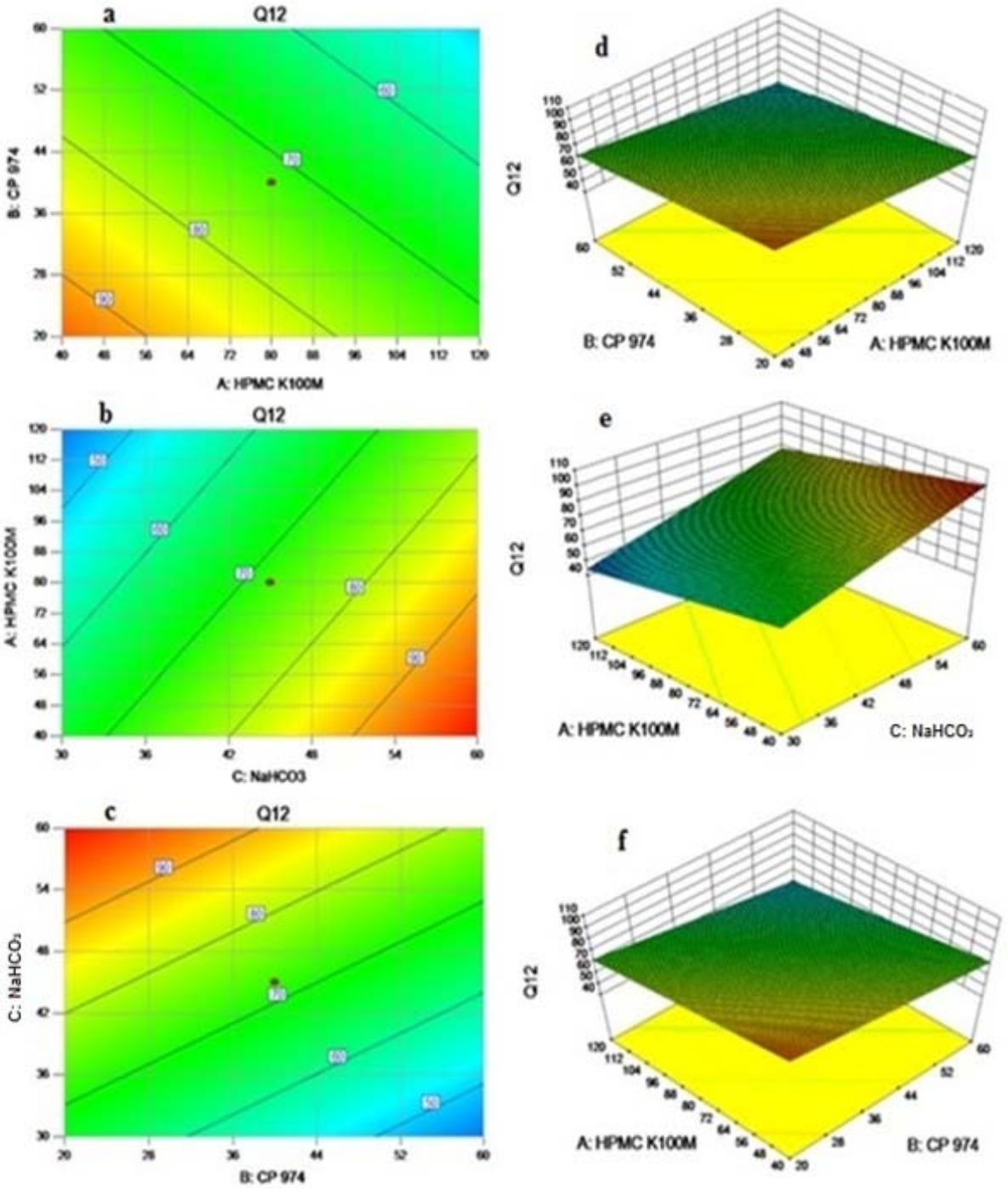

Figure 6: Contour plots showing effect of a) HPMC K100M (X_1) and CP 974P (X_2); b) HPMC K100M (X_1) and $NaHCO_3$ (X_3); c) CP 974P (X_2) and $NaHCO_3$ (X_3) on response Y_3 (drug released at 12 h); Corresponding response surface plots (d-f).

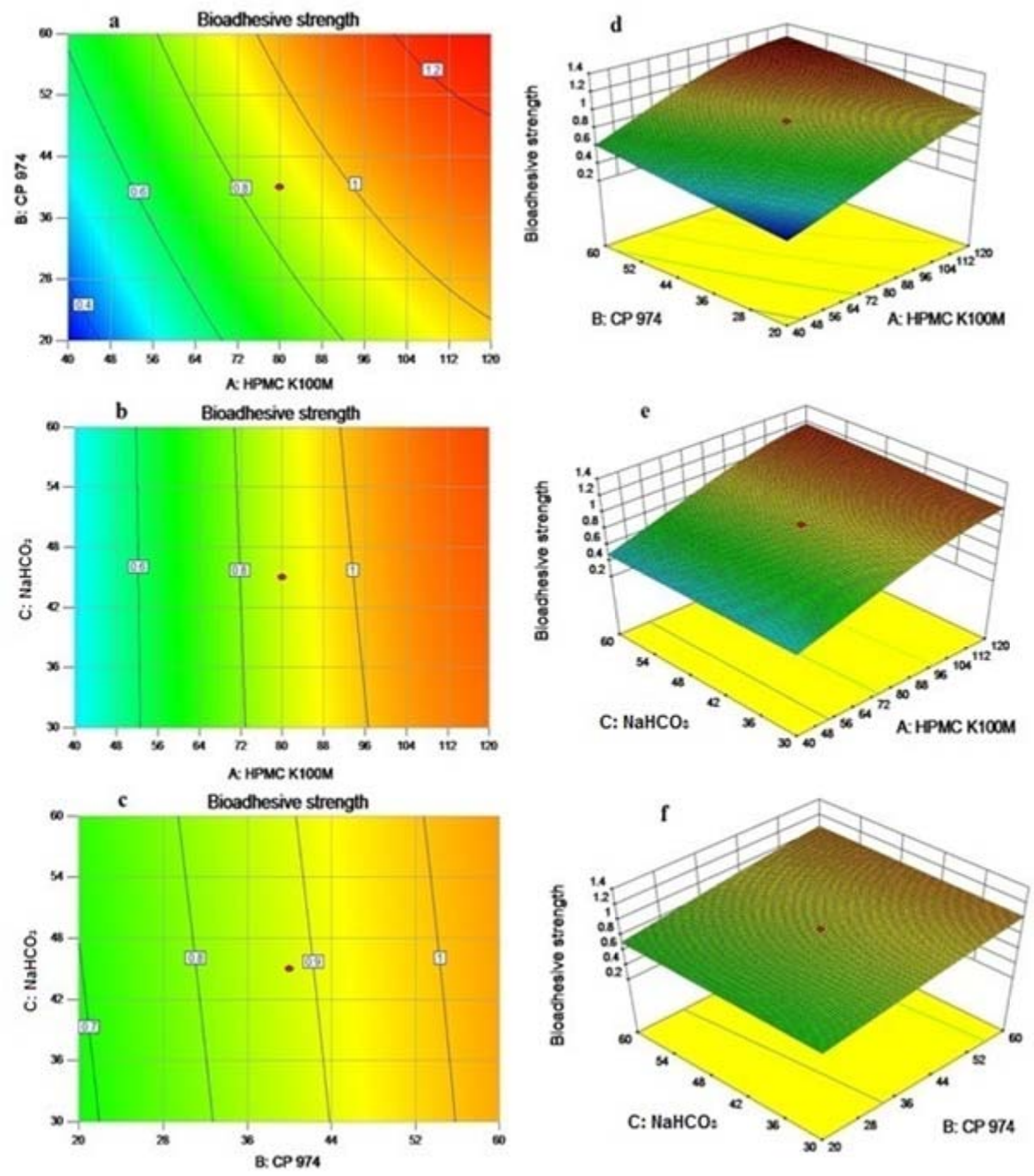

Figure 7: Contour plots showing effect of a) HPMC K100M (X_1) and CP 974P (X_2); b) HPMC K100M (X_1) and $NaHCO_3$ (X_3); c) CP 974P (X_2) and $NaHCO_3$ (X_3) on response Y_6 (Bioadhesive strength); Corresponding response surface plots (d-f).

Table 5: Comparison of predicted and experimental values of RSFBTsopt.

Response	Predicted values	Experimental values	Relative error (%)
Q_2 (%)	29.521	30.725	-4.078
Q_6 (%)	57.646	60.912	-5.665
Q_{12} (%)	90.002	97.253	-8.056
FLT (sec)	20.157	20.833	-3.353
TFT (h)	12.159	12.00	1.307
BS (N)	0.999	0.996	0.300

Table 6: Stability studies of RSFBTsopt.

Characteristic	0 day *	15th day *	30th day *	60th day *	90th day *
Hardness (kg/cm^2)	6.04±0.34	6.02±0.32	6.02±0.40	5.98±0.38	5.98±0.41
Drug content (%)	99.52±1.34	99.50±1.56	99.34±1.49	99.16±1.75	99.10±1.73
Floating lag time (s)	20.83±4.66	20.92±4.28	20.96±4.17	21.11±4.21	21.18±4.71
Duration of floating (h)	>12	>12	>12	>12	>12
Drug released at 12 h (%)	97.25±1.48	97.22±1.62	97.20±1.82	97.18±1.71	97.07±1.52

*The difference was not statistically significant ($p > 0.05$)

In vivo buoyancy study

The aim of this study was to examine whether the RSFBT could be buoyant and retained in the stomach. The *in vivo* buoyancy of tablet was confirmed by X-ray imaging at different time intervals post-administration of the $BaSO_4$-loaded RSFBTsopt. Figure 10 showed the gastric retention of $BaSO_4$-loaded RSFBTsopt in one volunteer. The first radiographic image was taken at 1 h post-administration of tablet and the tablet was seen in the stomach. In the next pictures (2.5 h and 4 h), it was observed that the tablet appeared more or less at the same position in the stomach. Later on, the tablet slightly changed its position and still remained within the stomach for 5.5 h. The mean gastric retention time was found to be 5 ± 0.86 h (n=3).

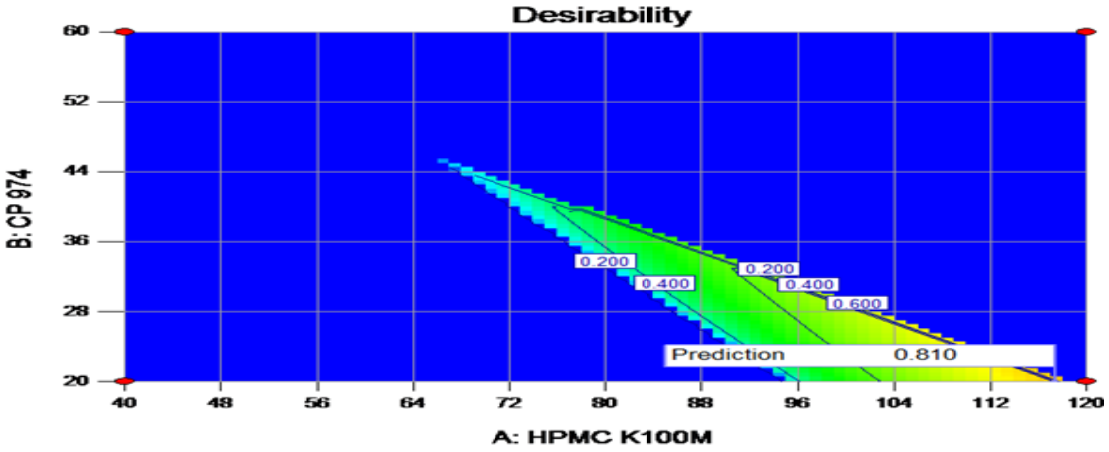

Figure 8: Desirability for optimization of RSFBT.

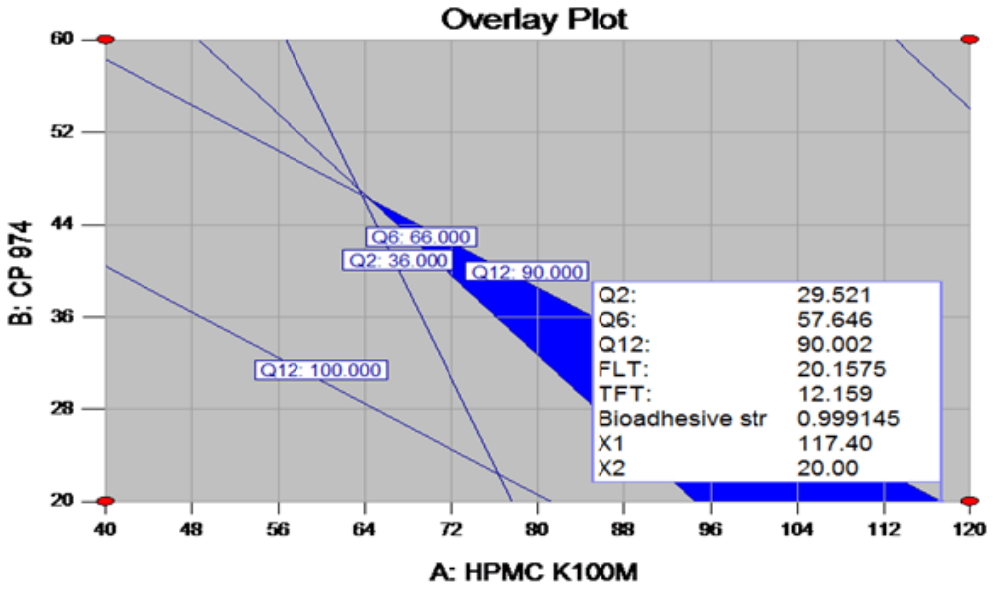

Figure 9: Overlay plot for optimization of RSFBT.

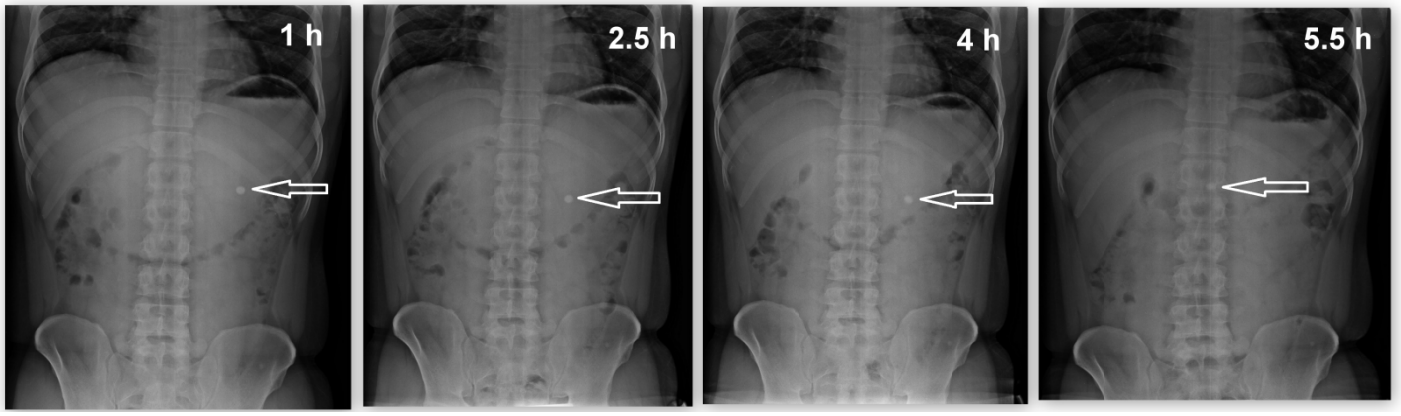

Figure 10: Radiographic images showing the presence of $BaSO_4$-loaded RSFBTsopt in the stomach at different time intervals (the tablet location was pointed out with a circle) of 1 h, 2.5 h, 4 h and 5.5 h.

Conclusion

The optimization of RSFBT formulation was carried out by CCD using DOE software. The optimized formulation (RSFBTsopt) was prepared using 117.4 mg of HPMC K100M (X_1), 20 mg of CP 974P (X_2) and 60 mg of $NaHCO_3$ (X_3), and showed the responses with respect to 30.73 ± 1.21 % Q_2 (Y_1), 61.96 ± 0.86 % Q_6 (Y_2), 97.25 ± 1.48 % Q_6 (Y_3), 21.17 ± 4.58 sec FLT (Y_4), greater than12 h TFT (Y_5), and 0.996 ± 0.032 BS (Y_6). These experimental response values were in close agreement with the predicted values confirming the predictability and validity of the model. The RSFBTsopt followed Peppas kinetic model with non-Fickian diffusion mechanism. Swelling studies indicated significant water uptake and contributed in drug release kinetics. Further, the RSFBTsopt formulation loaded with $BaSO_4$ in radiological studies in healthy volunteers, exhibited 5 ± 0.86 h (n=3) mean gastric residence time indicating the retention in gastric environment.

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Conflict of interest

The authors declare that there is no conflict of interest.

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