Original Research Article

Statistical optimization of floating pulsatile drug delivery system for chronotherapy of hypertension

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Abstract

Introduction: A pulsatile drug delivery system is characterized by a lag time that is an interval of no drug release followed by rapid drug release. The purpose of this work was to develop hollow calcium alginate beads for floating pulsatile release of valsartan intended for chronopharmacotherapy. Floating pulsatile concept was applied to increase the gastric residence of the dosage form having lag phase followed by a burst release. **Materials and Methods:** To overcome the limitations of various approaches for imparting buoyancy, hollow/porous beads were prepared by simple process of acid-base reaction during ionotropic crosslinking by low viscosity sodium alginate and calcium chloride as a crosslinking agent. In this study, investigation of the functionality of the sodium alginate to predict lag time and drug release was statistically analyzed using the response surface methodology (RSM). RSM was employed for designing of the experiment, generation of mathematical models and optimization study. The chosen independent variables, i.e. sodium alginate and potassium bicarbonate were optimized with a 3^2full factorial design. Floating time and cumulative percentage drug release in 6 h were selected as responses. **Results:** Results revealed that both the independent variables are significant factors affecting drug release profile. A second-order polynomial equation fitted to the data was used to predict the responses in the optimal region. The optimized formulation prepared according to computer-determined levels provided a release profile, which was close to the predicted values. The floating beads obtained were porous (21-28% porosity), hollow with bulk density <1 and had Ft_{70} of 2–11 h. The floating beads provided expected two-phase release pattern with initial lag time during floating in acidic medium followed by rapid pulse release in phosphate buffer. **Conclusion:** The proposed mathematical model is found to be robust and accurate for optimization of time-lagged formulations for programmable pulsatile release of valsartan.

Key words: Calcium alginate beads, chronopharmacotherapy, floating-pulsatile drug delivery, full factorial design, response surface methodology

INTRODUCTION

Natural biodegradable polysaccharides like pectin, guargum, chitosan, carrageenans, sodium alginate, and gellangum have been used in controlled drug delivery.[1-5] Multiparticulate systems obtained by ionotropic crosslinking of these polymers have been used to develop floating drug delivery. Several approaches are currently utilized in the prolongation of the gastric retention time, including floating drug delivery systems (FDDS), also known as hydrodynamically balanced systems (HBS), swelling and expanding systems, polymeric bioadhesive systems, modified-shape systems, high-density systems, and other delayed gastric emptying devices. These FDDS may be effervescent or non-effervescent. Various approaches to induce buoyancy in crosslinked beads, some of which include freeze-drying, entrapment of gas or gas forming agents, use of volatile or fxed oils, have been used.[6-8] These approaches are complicated as they require specifc equipment and handling techniques with limited acceptance. The oil-containing beads have limitations of coalescence of oil droplets yielding beads of wider particle size distribution, volatilization, or leaching of oil.[9] Comparatively, the floating dosage forms containing sodium bicarbonate as buoyancy imparting agent are simple to produce, which have been already attempted.[10,11] The floating property is based on the evolution of carbon dioxide when in contact with acidic environment followed by the ability of polymer gel to entrap it, which decreases their density below one. On the other hand, violent gas generation, disintegration of dosage form, burst release, dose dumping and alkaline micro environment [12] are limitations of these dosage forms. Choi *et al.*[13] have developed porous alginate beads containing riboflavin where the carbon dioxide gas was allowed to generate during

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crosslinking only, followed by freeze-drying to improve porosity. Talukder and Fassihi[14] developed a floatable multiparticulate system by crosslinking low methoxylated pectin and sodium alginate. The beads obtained by freeze-drying remained buoyant over 12 h, whereas the air-dried beads remained submerged. The study revealed the presence of air-flled hollow spaces inside the freeze-dried beads, which was responsible for the flotation property of the beads. Sriamornsak *et al*.[15] developed floating calcium pectinate beads by emulsion-gelation method. Such technique can be considered as an alternative to overcome limitations of sodium bicarbonate containing FDDS. Chronopharmacotherapy, the drug regime based on circadian rhythm, is recently gaining much attention worldwide. Various diseases like asthma, hypertension, acidity, and arthritis show circadian variation, which demands time-scheduled drug release for effective drug action, e.g., infammations associated with morning body stiffness, asthma, and heart attack in early hours of the day.[16] To follow this principle one must have to design the dosage form such that it can be given at the convenient time, e.g., bed time for the above-mentioned diseases with the drug release in the morning. Drug pharmacokinetics too show circadian variation for variousanti-infammatory drugs like indomethacin, ketoprofen, and diclofenac sodium, which have greater absorption in morning as compared to evening,[17] and site-specifc absorption from small intestine.[18,19] Therefore, to develop dosage form for chronopharmacotherapy the desired drug release should be time- as well as site-specifc.

Response surface methodology (RSM) is a collection of statistical and mathematical techniques useful for developing, improving, and optimizing processes.[20] The basic components of the methodology include various types of experimental designs, regression analysis, and optimization algorithms, which are used to investigate the empirical relationship between one or more measured responses and a number of independent variables in the form of polynomial equations and mapping of the response over the experimental domain, with the ultimate goal of obtaining an optimal problem solution and establishing the robustness of the process. The advantage of such methodology is in providing a rationale for simultaneous evaluation of several variables with minimum experimentation and time, thus proving to be far more efficient and cost effective than conventional methods of product development.

The purpose of the present study was to produce hollow/porous-floating beads of alginate by a process of evolution of carbon dioxide during crosslinking in acidic environment. Optimize method of preparation by statistical design so as to reduce the number of experiments required for optimization of formulation. Valsartan, an acid-insoluble anginotensin II antagonist, was used as model drug. The obtained beads were evaluated for entrapment efficiency, porosity, *in vitro* floating properties and *in vitro* drug release.

MATERIALS AND METHODS

Materials

Valsartan was obtained as gift sample from Lupin Pharmaceuticals Pvt. Ltd. Pune, India, as a gift sample. Sodium alginate was purchased from Loba Chemicals, Mumbai, India. Other excipients used to prepare beads were of standard pharmaceutical grade and all chemical reagents used were of analytical grade.

Methods

Experimental design[21-23]

A 3^2 full factorial design was constructed where the amounts of sodium alginate (X_1) and potassium bicarbonate (X_2) were selected as the two independent variables. It is suitable for investigating the quadratic response surfaces and for constructing a second-order polynomial model, thus enabling optimization. The levels of the two factors were selected on the basis of the preliminary studies carried out before implementing the experimental design. All other formulation and processing variable were kept constant throughout the study. Optimization of preparation of beads was done by Design Expert Software (Version 7.1.6, Stat-Ease Inc., and Minneapolis, MN). All the above formulations were prepared and evaluated for various parameters, and the effects of the polymers were studied on the dissolution test. The data was inputted to design expert software and polynomial equation was obtained. The responses (dependent variables) studied were cumulative percentage drug release at 6 h (Y1) and floating time (the time required to float 70% beads) (Y2). Table 1 summarizes the independent and dependent variables along with their levels. The resulted formulations (testing runs) are listed in Table 2.

Preparation of calcium alginate beads

Sodium alginate was added in distilled water and stir well then valsartan(150mg) which is dissolved in small amount of methanol was add in 10 ml of above prepared sodium alginate solution. As mentioned in Table 3, potassium hydrogen carbonate was added in various amounts. The dispersion was sonicated for 30 min. to remove any air bubbles. The resultant dispersion was dropped

Table 1: Translation of the coded levels in actual units

Coded level	Actual value in %	
	X1	X2
-1	4	0.5
0	5	0.625
1	6	0.75

Table 2: 3^2 full factorial design layout, experimental runs and their combinations

Batch No	X1	X2
F1	-1	-1
F2	-1	0
F3	-1	1
F4	0	-1
F5	0	0
F6	0	1
F7	1	-1
F8	1	0
F9	1	1

Table 3: Batch code and composition of each batch

Batch code	F1	F2	F3	F4	F5	F6	F7	F8	F9
Valsartan (mg)	150	150	150	150	150	150	150	150	150
Sodium alginate (% w/v)	4	4	4	5	5	5	6	6	6
Potassium bicarbonate ($KHCO_3$: Sodium alginate)	0.5	0.625	0.75	0.5	0.625	0.75	0.5	0.625	0.75
Calcium chloride (%w/v)	2	2	2	2	2	2	2	2	2
Acetic acid10% (v/v) in 100 ml $CaCl_2$ (2%)	10	10	10	10	10	10	10	10	10

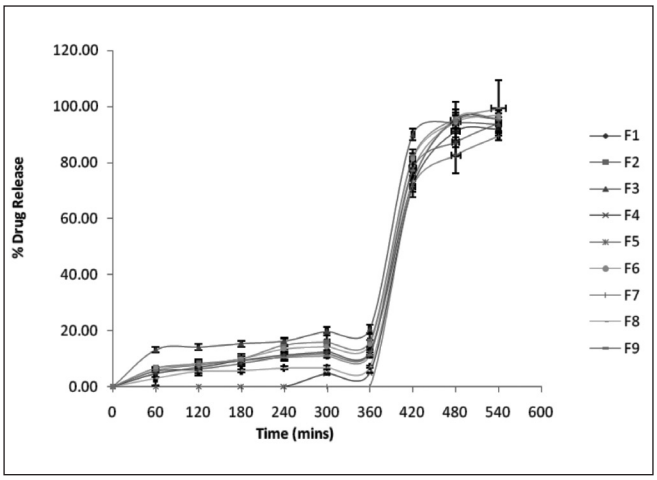

Figure 1: Cumulative % drug release profile of factorial design batches (F1-F9)

via a 24-gauge syringe needle from into 2% w/v calcium chloride solution ($CaCl_2$ solution) containing 10%(v/v) acetic acid. The content was stirred at 100-200 rpm using magnetic stirrer bar for 15 min. The beads were then filtered, washed three times with distilled water, and subsequently oven dried at 50º for 4 h.

Buoyancy test

The obtained beads were studied for buoyancy and floating time using USP 24 type II dissolution test apparatus (Electrolab DA-6D, Mumbai, India). 30 beads of each batch were placed in 900 ml of 0.1 N HCl (pH 1.2) containing 0.02%w/v tween 80 and agitated at 100 ± 5 rpm, temperature was maintained at 37º C ± 2.

Drug release studies

Dissolution studies were performed in triplicate using the USP dissolution test apparatus-II at 100 rpm. The dissolution studies of the beads equivalent to 40mg of valsartan were performed using USP 24 type II dissolution test apparatus (Electrolab DA-6D, Mumbai, India). The drug release study was carried out in 0.1 N HCl for initial 6 h followed with dissolution in phosphate buffer pH 7.4, each 900 ml, maintained at 37 ± 2 ºC and agitated at 100 rpm. Samples were collected periodically and replaced with a fresh dissolution medium. After filtration through muslin cloth, concentration of valsartan was determined spectrophotometrically (UV spectrophotometer (JASCO- V530, Kyoto, Japan) at 250 nm. Analysis of data was done using 'PCP Disso v2.08' software (Poona College of Pharmacy, Pune, India.)

Table 4: Dissolution and floating studies as per 3^2 full factorial experimental design

Formulation	% Drug release at 6 hr	Floating time (F_{t70}) in hr
F1	11.35	5
F2	15.29	7
F3	20.59	11
F4	7.18	3
F5	12.09	6
F6	16.29	9
F7	0	2
F8	4.95	5
F9	12.21	7

Statistical analysis of the data and validation of the model

Response surface modeling and evaluation of the quality to fit the model for the current study were performed employing Design Expert® software (Version 7.1.6, Stat-Ease Inc., Minneapolis, MN). Polynomial models including interaction and quadratic terms were generated for all the response variables using multiple linear regression analysis. 3D response plots were constructed using Design-Expert® software. One final formulations corresponding to the predicted optimum polymer concentration and three additional random check points covering the entire range of experimental domain were carried out to determine the validity of the model generated. Subsequently, the resultant experimental data of the response properties were quantitatively compared with those of the predicted values. Also, the linear regression plots between observed and predicted values of the response properties were drawn using MS-Excel.

RESULT AND DISCUSSION

Experiments of 3^2 full factorial design

To develop a system with time-lagged combined with floating delivery, the concentration of sodium alginate and potassium bicarbonate are important parameters affecting the drug release profle and floating time. A multi-variate optimization strategy was carried out with the aim of finding the optimum concentration to achieve a pulsatile release pattern. Figure 1 shows the release profiles of the 9 experimental runs performed in accordance with Table 3. Response data determined as per 3^2full factorial experimental design: response Y1 cumulative percentage drug release at 6 h and Y2 floating time (the time required to float 70% beads) are presented in Table 4.

Buoyancy study

Floating properties of beads was studied by determining buoyancy and time required for sinking all the beads under study.[24] The study was performed in 900 ml, 0.1N HCl (pH 1.2), containing 0.02%w/v Tween 80. The surfactant was used to simulate surface tension of human gastric juice (35-50mN/m^2). F_{t70} is time required to float 70% of beads. Batch F3 shows maximum f_{t70}, i.e., 11 h due to the high porous nature of beads. Batches F1 and F3 shows 5h and 11h f_{t70} resp. f_{t70} of batch F1, F4, and F7 is 5h, 3h, and 2h, respectively, indicating that floating time is significantly affected by potassium bicarbonate concentration as compared to sodium alginate concentration. The floating properties of hollow/porous beads may be attributed to the low bulk density and the porosity of the beads; implying that the beads will have the propensity to exhibit an excellent buoyancy effect *in vivo*.

In vitro drug release study

On the basis of *in vivo* gastric residence the floating beads were considered to be gastro retentive for 6 h making basis for *in vitro* dissolution time in acidic medium. All these beads released 0-20.59% of the drug in acidic medium irrespective of time. Batch F7 shows no drug release in acidic medium, while batch F4 and F8 shows minimum drug release 7.18% and 4.93%, respectively. After this lag, it was followed by pulse with complete drug release within 30-45 min in phosphate buffer [Figure 1]. The porous/hollow beads showed excellent lag in drug release at acidic pH that may be due to insolubility of drug and sodium alginate. At acidic pH calcium alginate remain protonated into insoluble form with reduced the swelling. The second phase of pulsed released in pH 7.4 can be attributed to rapid swelling and gel relaxation of calcium alginate gel at alkaline pH. Secondly, at pH > 6.6 valsartan is freely soluble that resulted in rapid and complete drug release. Hollowness/porosity of beads did not significantly affect drug release pattern in both mediums.

Multiple regression and mathematical model building

The targeted response parameters were statistically analyzed by applying one-way ANOVA (analysis of variance), at 5% significance level and the significance of the model was estimated using the statistical package Design-Expert. The individual parameters were evaluated using F-test and mathematical relationship was generated between the factors (dependent variables) and responses (independent variables) using multiple linear regression analysis, for determining the levels of factors which yield optimum dissolution responses. A second-order polynomial regression equation that fitted to the data is as follows:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 \quad (1)$$

Where b is the intercept representing the arithmetic averages of all the quantitative outcomes of 9 runs; b_1, b_2, b_{12}, $b_{11}$and b_{22} are the coefficients computed from the observed experimental values of Y; and X1and X2 stand for the main effects. The terms X1, X2 and Xi^2 (i = 1 and 2) represent the interaction and quadratic terms, respectively used to simulate the curvature of the designed sample space. In Table 5, factor effects of 3^2 FFD model and associated p-values for the responses Y1and Y2 are presented. A factor is considered to influence the response if the effects significantly differ from zero and the p-value is less than 0.05. Data in Table 5 show that significant factors affecting the response Y2 were synergistic effect of the linear contribution of main effects X1and X2 without producing any interaction. The response Y_2 was significantly affected by the antagonistic effect of linear contribution, quadratic contribution as well as cross-product contribution (interaction effects) of both the main effects $X_1$and X_2. A backward elimination procedure was adapted to fit the data into different predictor equations. The final equations of the responses given below,

% Drug release in 6h (Y1):+11.1-5.39*A+0.75*A*A+5.26*B-0.33*B*B+1.24*A*B-0.82*A*A*B-0.45*A4*B*B+0.57*A*A*B*B..... (2)

Floating time F_{t70} (Y2):+6.11-1.44*A-0.11*A*A+2.89*B-0.11*B*B-0.56 *A*B+0.11*A*A*B+0.44*A*B*B+0.11*A*A*B*B.....(3)

To confirm the omission of non-significant terms, an F statistic was calculated after applying analysis of variance for the full model and the reduced model. The equations represent the quantitative effect of factors ($X_1$and X_2) upon the responses (Y_1 and Y_2). Coefficients with one factor represent the effect of that particular factor while the coefficients with more than one factor and those with second order terms represent the interaction between those factors and the quadratic nature of the phenomena, respectively. Positive sign in front of the terms indicates synergistic effect while negative sign indicates antagonistic effect of the factors.

Search for optimum formulation

The results for the feasibility search to find suitable region for further location of optimum formulations were carried by using grid search. The criteria for selection of suitable feasible region were % drug release (0-15%) and floating time (6h).

Table 5: Results of ANOVA

Response model	Sum of square	Degree of freedom	Mean square	F value	*P* value	R square	Ade. precision
% drug release at 6h	309.36	4	77.34	79.95	0.0005	0.9876	28.06
Floating time (F_{t70})	61.78	4	15.44	55.60	0.0009	0.9823	22.062

The response surface diagrams, known to facilitate an understanding of the contribution of the variables and their interactions, and their respective contour maps are shown for all the responses in Figures 2 and 3.

Figures 2 and 3 revealed that both variables have significant effect on the % drug release but magnitude of X variable i.e. sodium alginate concentration is high as compared to Y variable i.e. concentration of potassium bicarbonate.% drug release decrease with increase in concentration of sodium alginate and vice versa for potassium bicarbonate. This is may be result of higher concentration of sodium alginate causes high intermolecular bonding and compactness of beads.

Figures 4 and 5 revealed that both variables have significant effect on the floating time but magnitude of potassium bicarbonate concentration is high as compared to concentration of sodium alginate. The floating time increases with increase in concentration of potassium bicarbonate and vice versa for sodium alginate. The floating properties of hollow/porous beads may be attributed to the low bulk density and the porosity of the beads.

The Model F-value for % drug release was found 79.95, which imply the model is significant. *P* value, which is 0.0005, was less than 0.0500 indicates model terms are significant. The signal to noise ratio of 28.06 indicates an adequate signal thus the proposed model can be used to navigate the design space.

For floating time (ft70), the Model F-value was found 61.78.80, which implies that the model is significant. *P* value, which is 0.0009, was less than 0.0500 indicates model terms are significant. The signal to noise ratio of 22.062 indicates an adequate signal thus the proposed model can be used to navigate the design space. Since the values of r^2 are quite high for both the responses, i.e., 0.9876 for % drug release and 0.9823 for floating time, the polynomial equations form excellent fits to the experimental data and are highly statistically valid.

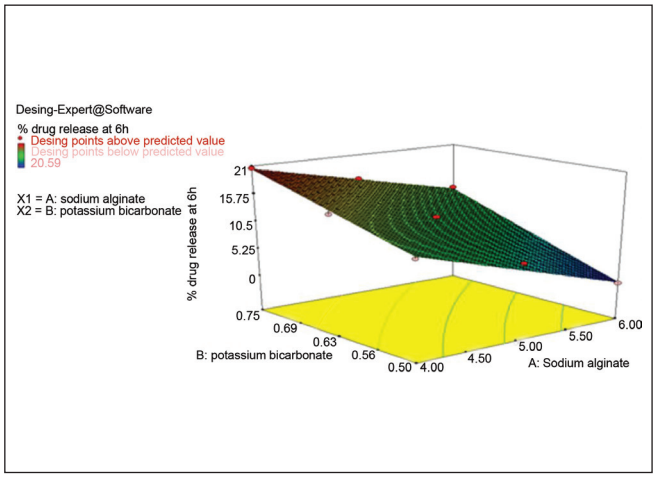

Figure 2: A contour plot showing relationship between various levels of independent variables to attain fixed values of % drug release

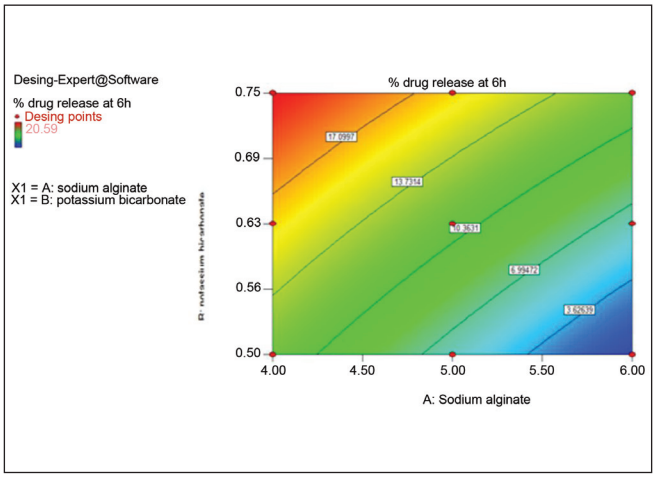

Figure 3: A response surface plot showing influence of concentration of independent variables on the % drug release

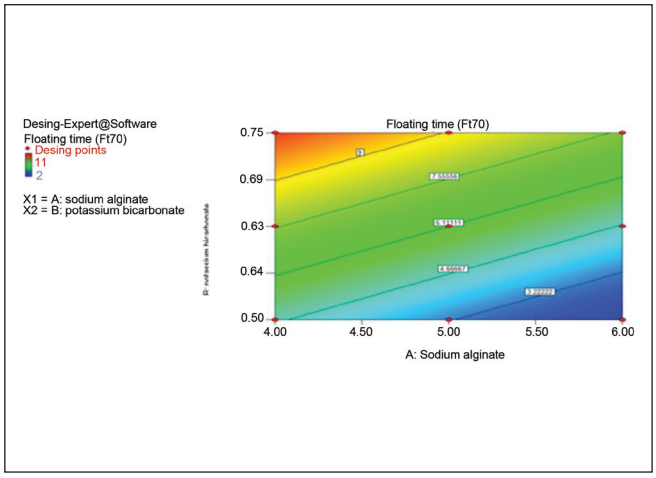

Figure 4: A contour plot showing relationship between various levels of independent variables to attain fixed values of floating time

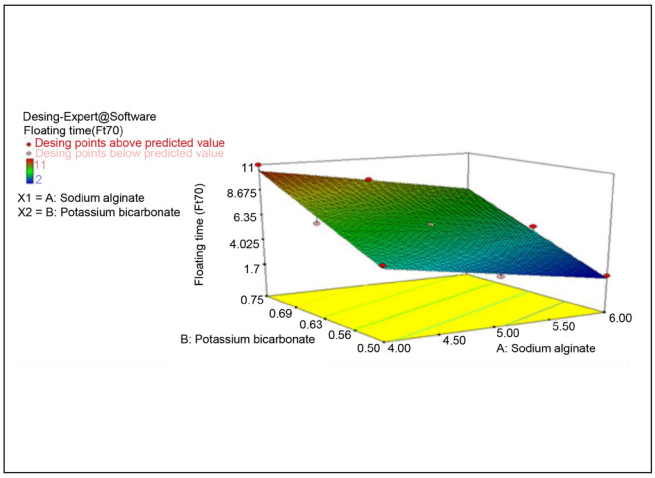

Figure 5: A response surface plot showing influence of concentration of independent variables on the floating time

Table 6: Validation of Response surface methodology

Batch Code	Composition		Response	Predicated value	Experimental value	Residuals	% Error
	X1 (g)	X2 (%)					
Q1	5	0.625	Ft70	6.11	6	0.11	1.8
			% Drug release	11.1	11.41	-0.31	2.7169
Q2	5.2	0.625	F_{t70}	5.81	6	-0.19	3.27
			% Drug release	10.052	10.28	-0.528	2.159
Q3	5.4	0.65	F_{t70}	6.05	5	1.05	10.54
			% Drug release	10.1722	10.10	0.0689	0.6773

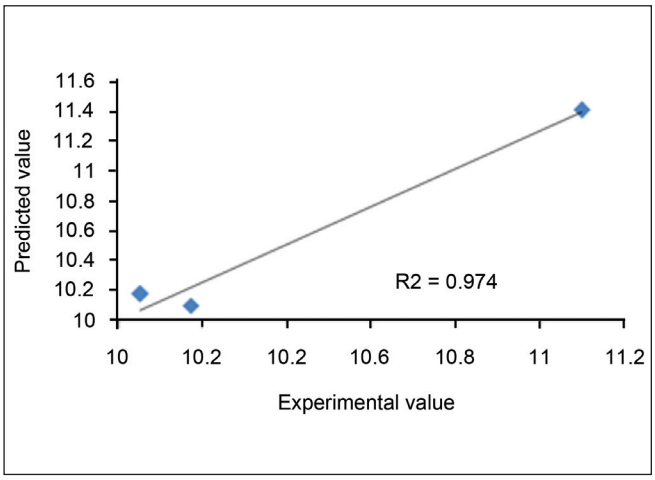

Figure 6: Linear plots between observed and predicted values of % drug release

Validation of RSM

Table 6 indicates the formulations as per the predicted responses prepared as per the optimum region from grid search, the values of % drug release at 6 h ranged between 10.10-11.41 % minimum % drug release from beads . The values of floating time ranged from 5 h to 6 h.

Comparative table of the observed responses with that of the predicted responses along with percentage error is listed in Table 6. The plots between the observed and the predicted responses were shown in Figure 6. The linear correlation plots drawn between the predicted and observed responses demonstrated good values of r^2 (0.974). Hence, the prognostic ability of the experimental design to predict % drug release and floating time of prepared hollow beads of valsartan is validated.

Upon comparison of the observed responses with that of the anticipated responses, the prediction error varied between -0.528 and 1.05. Thus, the low magnitudes of error as well as the significant value of r^2 in the current study indicate a high prognostic ability of beads formulations of valsartan using Response surface methodology (RSM).

CONCLUSION

RSM is an important tool for understanding the change of responses and locating the area of interest. The formulation containing sodium alginate 5.2% and potassium bicarbonate 0.625% was in the optimum zone and has the potential for time-controlled pulsatile delivery of Valsartan. The optimized formulation exhibited release profiles that were close to the predicted responses. High degree of prognosis obtained for 3^2 full factorial design corroborates that RSM is an efficient tool in optimization experiments. This work can be extended for time scheduled release of drugs having high solubility, and poor absorption. Thus, the designed device can be considered as one of the promising formulation technique for preparing floating pulsatile drug delivery systems and hence in chronotherapeutic management of hypertension.

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