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Research Article

In Situ Forming Formulation: Development, Evaluation, and Optimization Using 3^3 Factorial Design

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Abstract. The present investigation concerns with the development and optimization of an *in situ* forming formulation using 3^3 full factorial design experimentation. Metformin, an antidiabetic drug with upper part of gastrointestinal tract as absorption window was used as a model drug. The formulations were designed with an objective to retain in stomach for an extended time period. The effect of three independent factors—concentrations of sodium alginate (X_1), gellan gum (X_2), and metformin (X_3) on *in vitro* drug release were used to characterize and optimize the formulation. Five dependent variables—release exponent (Y_1), dissolution efficiency (Y_2), drug release at 30 min (Y_3), 210 min (Y_4), and 480 min (Y_5) were considered as optimization factors. The data were statistically analyzed using ANOVA, and a $p<0.05$ was considered statistically significant. Three dimensional surface response plots were drawn to evaluate the interaction of independent variables on the chosen dependent variables. Of the prepared 27 formulations, the responses exhibited by batch F17 containing medium level sodium alginate (X_1), low level gellan (X_2), and medium level metformin (X_3) were similar to the predicted responses.

KEY WORDS: alginate; diffusion; factorial design; *in situ* forming; metformin.

INTRODUCTION

Gellan gum (commercially available as Gelrite® or Kelcogel®) is an anionic deacetylated polysaccharide secreted by *Pseudomonas elodea.* Gellan has the characteristic property of cation-induced gelation wherein the gelation involves formation of double helical junction zones followed by aggregation of the double helical segments to form a three-dimensional network by complexation with cations and hydrogen bonding with water (1–3). Sodium alginate is a linear copolymer composed of two monomeric units, D-mannuronic acid, and L-guluronic acid and is widely used in pharmaceutical formulations in beads, matrix tablets, microcapsules, etc. Gelation of dilute solutions of sodium alginate occurs in presence of di- and trivalent metal ions by a process involving consecutive guluronic residues in the guluronic acid (G) blocks of the alginate chain (4–6). When formulated for use in sustained drug delivery, the alginate is usually in the form of a matrix (7,8).

Metformin is the only currently available oral antidiabetic or hypoglycemic agent which acts predominantly by inhibiting hepatic glucose release with an absolute oral bioavailability of 40% to 60% (9). Gastrointestinal absorption occurs mainly in the upper intestine with peak plasma concentrations (C_{max}) reaching after 2 to 3 h (10). In view of its physicochemical and pharmacokinetic properties, it is worth using metformin as a model drug in development of gastroretentive systems.

With progressive research for development of newer drug delivery systems, it has become apparent to assess the factors influencing formulation and drug release in a short time. It is very important to develop and optimize a formulation with a few experiments and at a low cost in order to overcome the rapidly increasing cost of experiments (11–14). Optimization of formulations using statistical experimental designs is a powerful and efficient tool in the development of pharmaceutical dosage forms. The experimental design allows for studying various processing parameters influencing the selected responses with the lowest number of experiments, thereby reducing the time required in the development work (15–18).

In situ gel forming formulations present a novel idea of delivering drugs to patients as a liquid dosage, yet achieve sustained release of drug for the desired duration. Although there have been a few reports on the development of *in situ* forming formulation, development of the system with gastroretentive property has been less reported. In a preliminary study, we had reported the formulation and evaluation of *in situ* forming system with floating characteristics (19).

The present study was designed with an objective of further evaluating the influence of concentration of three independent variables: sodium alginate, gellan gum, and metformin on various selected responses. Further, it was aimed at optimizing independent variables using statistically designed experiment to achieve selected dependent response

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equivalent to that of marketed product. The selected dependent responses were: release exponent (Y_1), dissolution efficiency (Y_2), drug release at 30 min (Y_3), 210 min (Y_4), and 480 min (Y_5). The constraints on dependent variables was, Y_3=21–26%, Y_4=62–65%, and Y_5=91–94%. The optimization target and the constraints were set based on the dissolution data of MELMET-SR, a commercially available tablet of metformin.

MATERIALS AND METHODS

Materials

Metformin was a gift from Ranbaxy Research Laboratories, Gurgaon, (India). Sodium alginate (viscosity of 2% *w/v* solution 250 cps at 25°C) was procured from Sigma chemicals, St Louis, MO, USA. Gellan gum (Kelcogel®) was procured from CP Kelco, San Diego, CA, USA. MELMET-SR tablets were purchased commercially from market for comparative study. All other reagents and chemicals were of analytical grade and used as received.

Methods

Preparation of Formulations

Solution of sodium alginate and gellan, alone, or in combination (Tables I and II) was made in deionized water. The solution was heated to 60°C with constant stirring on a magnetic stirrer (Remi Instruments Ltd. Mumbai, India). The solution was allowed to cool to 40°C, when the drug and calcium carbonate (1.5% *w/v*) were dissolved and dispersed, respectively, in the resulting solution and finally stored in amber color bottles until further use (20).

Experimental Design

The present study consisted of a three-level three-factorial (3^3) design for experimentation. Statistical experimental design was performed using a software DESIGN EXPERT® version 7.0.2.8 (Stat-Ease Inc., Minneapolis, USA). Response surface graphics were used to show the factor interaction between the considered variables. Selected independent variables studied were the concentration of sodium alginate (X_1); gellan gum (X_2); and metformin (X_3) added to the formulation. Three factorial levels coded for low, medium, and high settings (−1, 0 and +1, respectively) were considered for three independent variables (21,22). The selected dependent variables investigated were the "*n*" value (Y_1), dissolution efficiency at 8 h (Y_2), percentage of drug released at 30 min (Y_3), 210 min (Y_4), and 480 min (Y_5). The number of trials required for the study is based on the number of independent variables selected. A total of 27 experimental runs were required for analyzing the interaction of each level on formulation characters and to optimize. Tables I and II show the factors chosen and different factor level settings. The response (Y_i) in

Table I. 3^3 Full Factorial Design of *In Situ* Forming Formulation

	Variable in coded value			Independent variables[a]				
Batch	X_1	X_2	X_3	Y_1	Y_2 (%)	Y_3 (%)	Y_4 (%)	Y_5 (%)
F1	−1	−1	−1	0.47±0.03	74.88±1.96	35.79±0.73	73.78±1.19	98.29±1.46
F2	0	−1	−1	0.52±0.02	68.34±0.91	27.43±0.96	65.75±0.73	96.57±0.33
F3	+1	−1	−1	0.60±0.11	69.87±0.99	22.80±3.61	70.11±1.24	92.61±0.79
F4	−1	0	−1	0.50±0.04	71.77±1.08	32.55±2.90	67.24±0.69	94.46±1.24
F5	0	0	−1	0.52±0.05	69.51±1.85	26.06±1.45	62.17±0.48	89.81±1.22
F6	+1	0	−1	0.54±0.04	68.98±1.33	23.50±1.19	56.65±0.49	82.09±1.71
F7	−1	+1	−1	0.54±0.06	73.38±0.86	24.70±1.93	71.76±0.95	91.89±1.46
F8	0	+1	−1	0.57±0.03	73.32±1.41	22.14±1.20	67.11±1.19	86.28±1.92
F9	+1	+1	−1	0.56±0.03	67.04±1.95	20.94±0.95	51.47±1.93	80.80±0.05
F10	−1	−1	0	0.52±0.03	80.61±1.38	48.58±0.55	83.26±0.74	98.57±0.76
F11	0	−1	0	0.55±0.10	73.59±2.10	40.43±0.92	73.87±0.76	97.70±0.40
F12	+1	−1	0	0.55±0.02	71.78±1.83	37.15±0.73	66.75±1.48	94.93±0.40
F13	−1	0	0	0.44±0.02	71.19±1.55	32.86±0.56	68.47±0.57	95.58±0.53
F14	0	0	0	0.45±0.02	69.62±0.90	29.97±0.93	64.74±0.91	92.68±0.74
F15	+1	0	0	0.46±0.03	69.05±1.36	28.00±1.10	58.00±0.38	86.34±0.95
F16	−1	+1	0	0.44±0.01	71.77±1.44	29.06±1.11	69.10±1.46	94.12±0.74
F17	0	+1	0	0.49±0.05	68.58±1.87	25.25±0.92	64.05±1.88	93.70±1.16
F18	+1	+1	0	0.48±0.01	66.58±1.34	24.32±0.74	54.12±0.92	84.36±0.57
F19	−1	−1	+1	0.59±0.02	80.85±1.93	41.27±1.04	84.64±0.72	99.52±0.73
F20	0	−1	+1	0.62±0.03	78.35±1.46	37.09±0.98	77.12±1.87	94.75±0.95
F21	+1	−1	+1	0.62±0.01	74.91±1.09	25.31±0.64	70.61±0.98	91.64±0.87
F22	−1	0	+1	0.52±0.02	77.05±1.30	37.45±3.39	75.00±2.04	97.23±1.58
F23	0	0	+1	0.54±0.02	74.87±1.52	30.82±0.80	70.22±0.61	93.09±0.85
F24	+1	0	+1	0.59±0.02	70.34±1.47	22.82±0.99	59.64±0.21	87.54±1.43
F25	−1	+1	+1	0.67±0.04	72.37±1.05	35.93±0.70	73.33±0.99	98.12±1.07
F26	0	+1	+1	0.66±0.03	73.68±1.34	32.08±0.99	70.91±1.21	94.93±0.65
F27	+1	+1	+1	0.58±0.01	67.56±1.69	31.52±0.59	60.37±0.80	88.57±0.42

[a] n=3, Mean±SD
X_1 sodium alginate; X_2 gellan gum; X_3 metfomin

Table II. 3^3 Full Factorial Design of *In Situ* Forming Formulation

	Actual values		
Coded value	X_1 (% *w/v*)	X_2 (% *w/v*)	X_3 (% *w/v*)
High (+1)	2.25	0.50	5.0
Medium (0)	1.75	0.25	3.75
Low (−1)	1.25	0	2.5

All batches contained 1.5% *w/v* of calcium carbonate
X_1 sodium alginate; X_2 gellan gum; X_3 metfomin

each trial was measured by carrying out a multiple factorial regression analysis using the quadratic model:

$$Y_i = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_1X_2 + b_5X_1X_3 + b_6X_2X_3 + b_7X_1^2 + b_8X_2^2 + b_9X_3^2$$

Where Y_i is the dependent variable; b_0 is the arithmetic mean response of all trials; and b_i is the estimated coefficient for factor X_i. The main effects, X_1, X_2, and X_3, represent the average value of changing factor one at a time; X_1X_2, X_1X_3, and X_2X_3 represent the interaction terms and the polynomial terms (X_1^2 , X_2^2 , and X_3^2) are used to assess nonlinearity.

In Vitro Gelation Study

The gelation studies were carried out in gelation cells, fabricated locally using Teflon®. The cells were cylindrical reservoirs capable of holding 10 mL of simulated gastric fluid as gelation solution (0.1 N HCl, pH1.2). Within the cells located at the bottom is a transparent plastic cup to hold the gel sample in place after its formation. Two milliliters of the formulation was carefully placed into the cavity of the cup using a micropipette, and 6 mL of the gelation solution (SGF) was added slowly and the rate of gelation was detected by visual examination**.**

In Vitro Buoyancy of In Situ Formed Gel

In vitro buoyancy lag time and duration was determined in a USP XXIII dissolution apparatus-II with 900 mL of SGF (0.1 N HCl, pH1.2) at 37±2°C with a paddle speed of 50 rpm. A measured sample of 10 mL was placed on a Petri dish, which was placed in the dissolution medium. The time taken by the gelled mass to reach to the top of the dissolution medium (onset of floating), and the duration of floating were noted.

In Vitro Drug Release Studies

The release of metformin from the gelled mass was determined as described by Zatz and Woodford (23) with minor modification using USP XXIII dissolution test apparatus (Cintex instruments, Mumbai, India) with a paddle speed at 50 rpm. Simulated gastric fluid (900 mL; 0.1 N HCl, pH1.2) maintained at 37±2°C was used as dissolution media. Ten milliliters of the formulation was transferred on to a Petri dish using a disposable syringe, and the dish containing formulation was placed in the dissolution vessel, carefully added dissolution fluid without much disturbance. At a pre-identified time interval, an aliquot was removed and replenished with fresh medium. The samples were assayed for metformin at 233 nm using spectrophotometer (Jasco 7800, Japan) after suitable dilution. A concurrent dissolution was performed with preparation devoid of drug to record the interference from excipients, if any. All the studies were conducted in triplicate, and the average was recorded.

Mechanism of Drug Release

The drug release data were fit to Korsmeyer–Peppas' power equation (24,25):

$$M_t/M_\infty = Kt^n$$

Where, M_t/M_∞ is the fraction of drug released in time t, K is constant, and n represents the release exponent indicative of mechanism of drug release. When n=0.5 means Fickian diffusion, $0.5>n<1.0$ non-Fickian diffusion, and n=1.0 Case II diffusion (25).

RESULTS

Preparation and Characterization of the Formulation

The preparation of *in situ* forming system is simple, reliable, and reproducible involving dissolving of metormin in polymeric solution (sodium alginate and gellan). Different formulations were prepared at various coded values for drug, sodium alginate, and gellan (Tables I and II). However, for ease of administration and handling, the preparation remains as liquid formulation but on ingestion undergoes gelation due to the availability of liberated Ca^{2+}. Excepting a few, formulations have shown an instantaneous gelation (<60 s). Among the others, the time required for complete gelation was 2–5 min. The buoyancy study was done in simulated gastric fluid (0.1 N HCl, pH1.2). Buoyancy lag time varied from 3 to 9 min, and the duration of buoyancy was >24 h irrespective of various formulation variables. The buoyancy lag time decreased with increasing concentration of calcium carbonate. However, increasing of calcium carbonate concentration >1.5% *w/v* did not impart any marked change in buoyancy lag time. Therefore, for further studies, 1.5% *w/v* calcium carbonate was used.

In Vitro Drug Release Studies

A preliminary study was conducted to optimize the concentration of $CaCO_3$. A few formulations containing calcium carbonate (1–2% *w/v*) was prepared to investigate its effect on drug release from the formulation. The insoluble calcium carbonate solubilizes in acidic pH, releasing the calcium ions and carbon dioxide. The study had shown that the drug release from the formulation decreased with increase in the concentration of calcium carbonate. Formulation containing 1% *w/v* $CaCO_3$ was completely depleted of drug (~99.07±1.45%) within 6 h; however, $CaCO_3$ at 1.5% *w/v* sustained the drug release as only 89.12±0.38% of drug was released at the end of 8 h dissolution study. Further increment in $CaCO_3$ (2% *w/v*) did not alter drug release significantly ($p>0.05$).

Drug release profiles from the formulations at various concentrations of sodium alginate are shown in Figs. 1, 2, 3, and 4. The drug release deceased with increase in the

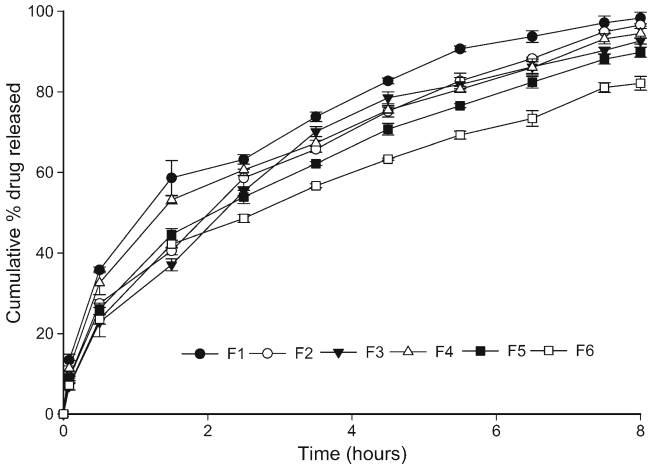

Fig. 1. Drug release profiles from the formulations (*F1–F6*) prepared following 3^3 factorial experimentation

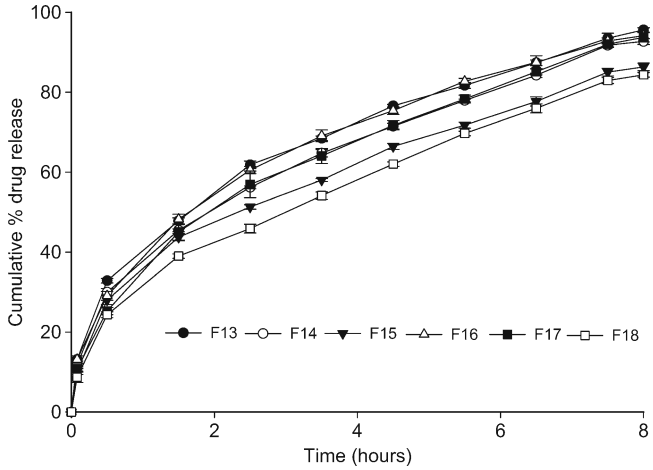

Fig. 3. Drug release profiles from the formulations (*F13–F18*) prepared following 3^3 factorial experimentation

concentration of sodium alginate. Formulations contained sodium alginate and gellan as anionic polymers. The influence of gellan on drug release can be observed from the drug release profiles. Incorporation of gellan gum as another source of anionic polymer exhibited a decrease in the burst effect. The drug release decreased as gellan to sodium alginate ratio was increased. The formulation contained metformin at three levels, i.e., low (2.5% *w/v*), medium (3.75% *w/v*), and high (5% *w/v*). As evident from different drug release profiles shown (Figs. 1, 2, 3, and 4), drug release increased with increase in the drug loading.

Mechanism of Drug Release

The mechanism of drug release was determined using Korsemeyer–Peppas equation (24,25). The release exponent (n) for all the formulations was in the range of 0.44±0.02–0.67±0.04 (Table I).

Experimental Designing and Analysis of Variance

A 3^3 full factorial design was used to investigate effect of three factors-sodium alginate, gellan, and drug loading-on drug release from *in situ* forming systems of metformin**.** The factorial design was carried out using the software DESIGN EXPERT® version 7.0.2.8 (Stat-Ease Inc., Minneapolis, USA). Table I shows the data obtained for the experiment trails after the factorial design.

Analysis of variance (ANOVA) was applied for estimation of significance of the model. Using a 5% significance level, a model was considered significant if the $p<0.05$. It was found that for responses Y_1 and Y_2, quadratic contribution and linear contribution model were significant ($p<0.001$), respectively. Response Y_3 showed more significant linearity to both linear and cubic contribution model. However, for responses Y_4 and Y_5, all models except 2F1 and cubic contribution model, respectively, were significant.

Estimation of Quantitative Effects of Factors

ANOVA was performed for estimation of quantitative effects of the factors. Response surface regression analysis was performed using coded values of factor levels (−1, 0, +1) for each factor to determine the significance. Table III shows the factor effects of the quadratic model and associated p values for all five responses. Figures 5, 6, and 7 show the

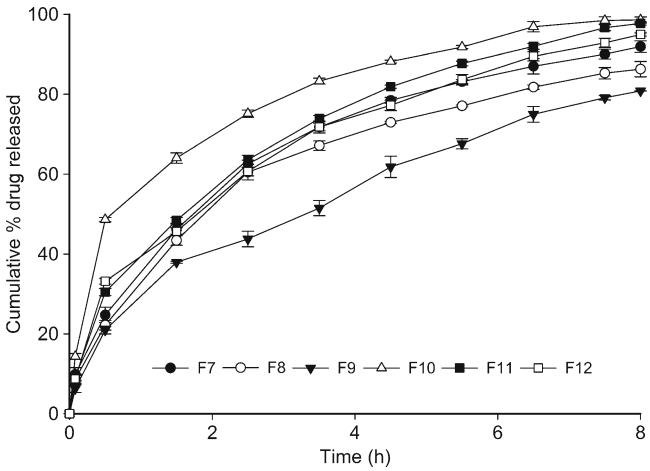

Fig. 2. Drug release profiles from the formulations (*F7–F12*) designed according to 3^3 factorial experimentation

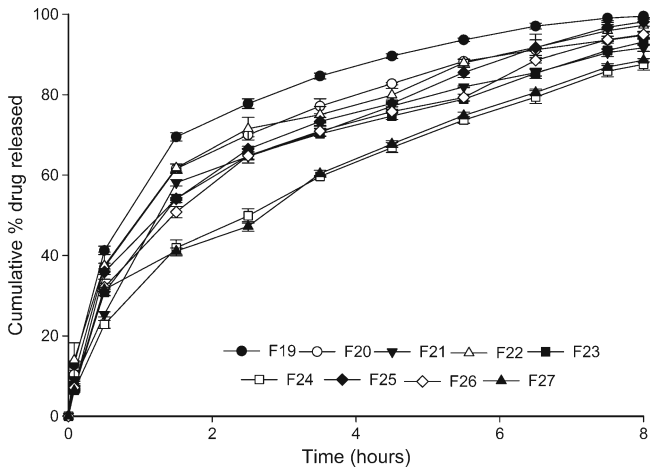

Fig. 4. Drug release profiles from the formulations (*F19–F27*) prepared following 3^3 factorial experimentation

Table III. The Quantitative Factor Effects and Associated *P* Value for all Five Responses

	Y_1		Y_2		Y_3		Y_4		Y_5	
Factor	Factor effect	*p* Value	Factor effect	*p* Value	Factor effect	*p* Value	Factor effect	*p* Value	Factor effect	*p* Value
X_1	+0.016	0.0687	−2.66	<0.0001	−4.52	<0.0001	−6.59	<0.0001	−4.38	<0.0001
X_2	−4.111E−003	0.6266	−2.16	<0.0001	−4.37	<0.0001	−4.65	<0.0001	−2.86	<0.0001
X_3	+0.032	0.0004	1.83	<0.0001	2.77	<0.0001	3.10	<0.0001	1.83	<0.0001
X_1X_2	−0.020	0.0587	0.29	0.4478	2.32	0.0012	−1.17	0.0487	−1.10	0.0014
X_1X_3	−0.015	0.1483	−0.28	0.4637	−0.70	0.3013	−0.65	0.2631	0.17	0.5986
X_2X_3	+3.333E−004	0.9743	−1.76	<0.0001	0.47	0.4827	−0.70	0.2277	2.05	<0.0001
X_1^2	−7.833E−003	0.5926	0.011	0.0211	0.37	0.6945	−0.97	0.2387	−1.29	0.0012
X_2^2	+0.051	0.0012	1.27	0.0211	1.38	0.1510	4.64	<0.0001	2.23	<0.0001
X_3^2	+0.079	0.0001	1.20	0.0282	−3.89	0.0002	1.27	0.1269	−0.96	0.0407

response surface plots of the effect of sodium alginate (X_1), gellan (X_2), and metformin (X_3) on the dependent variable Y_5.

DISCUSSION

Preparation and Characterization of the Formulation

The preparation of *in situ* forming system being simple and reliable showed an excellent reproducibility. In this formulation, sodium alginate and gellan were used which, on gelation, forms a matrix barrier and sustain drug release. The calcium ions interact with anionic polymer (alginic acid and/or gellan) in the formulation causing instantaneous gelation; during this process, the liberated CO_2 is entrapped within the gel matrix attributing to the buoyancy of the mass (26). Both $CaCO_3$ and $NaHCO_3$ have been reported as gas-generating agents in formulations (26). However, in this study, $NaHCO_3$ was excluded since it is soluble in neutral pH giving rise to Na^+ which induces premature gelation of alginate and gellan gum.

In Vitro Drug Release Studies

On the basis of the preliminary study, concentration of calcium carbonate was fixed at 1.5% *w/v*. Drug release was not significantly modified when the concentration of calcium carbonate was increased to 2% *w/v*. This critical calcium level may corresponds to a state in which the number of calcium ions was barely adequate to link the anionic sites in the junction's zones and, thus, increase the gel strength of the gel (26). Above that level, additional ions may occupy the anionic sites, linkages forming between adjacent polymer chains, and introduce repulsive forces in the junction zone resulting in weakened gel structure. Therefore, for further studies, the calcium carbonate level was fixed at 1.5% *w/v*.

The observed decrease of percentage drug release from the alginate gels with increase of alginate concentration is due to the concomitant increase in gel strength (27). *In vitro* release of metformin from gel was determined in simulated gastric fluid (0.1 N HCl, pH1.2).When formulation containing sodium alginate comes in contact with SGF, the calcium carbonate break down and releases free Ca^{2+} ions that induce gelation due to dimeric association of G-block regions of sodium alginate (5). The controlled availability of calcium ions leads to formation of insoluble alginate gel formation (28). With low levels of calcium, temporary associations are obtained, giving rise to highly viscous, thixotropic solutions. At higher calcium levels, precipitation or gelation results from permanent associations of the chains. Similar to aliginic acid, the reactions also proceeds with anionic gellan gum giving rise to gel matrix. Gellan, an exopolysaccharide, was incorporated as an anionic polymer in the formulation. It being anionic readily cross-links with free calcium ions. The observed decrease in drug release with increase in gellan to sodium alginate ratio could be explained by the fact that the diffusion coefficient through gellan is of similar magnitude to that of sodium alginate despite the use of gellan in smaller proportion (20). With increase in drug loading, the matrix of gel formed would become more relaxed allowing easy solvent penetration leading to enhanced drug diffusion (29); this could be the reason for increased drug release while increasing drug concentration.

Dissolution efficiency (i.e., area under the dissolution curve between 0 to time *t*) decreased with increase the concentration of sodium alginate and gellan (Tables I and II). Highest percentage dissolution efficiencies, 80.61 ± 1.38% and 80.85 ± 1.93%, were observed for batches F10 and F19, respectively. This may be due to low concentration of sodium alginate, and formed gel may be weak or not sufficient to sustain the release of high dose of metformin. The combination of sodium alginate and gellan caused about a 14% decrease of dissolution efficiency in relation sodium alginate alone (~93.73%).

Mechanism of Drug Release

Metformin release from the formulations showed linearity towards Higuchi's square root model (30), indicating the release mechanism to be diffusion based. The release exponents (n) of the formulations suggest that depending on the formulation variables the metformin release followed either Fickian or non-Fickian mechanism. The drug diffusion through most types of polymeric systems is often best described by Fickian diffusion, but there is also a relaxation of the polymer chains, which influences the drug release mechanism. This process is described as non-Fickian or anomalous diffusion (29). The observed deviation from

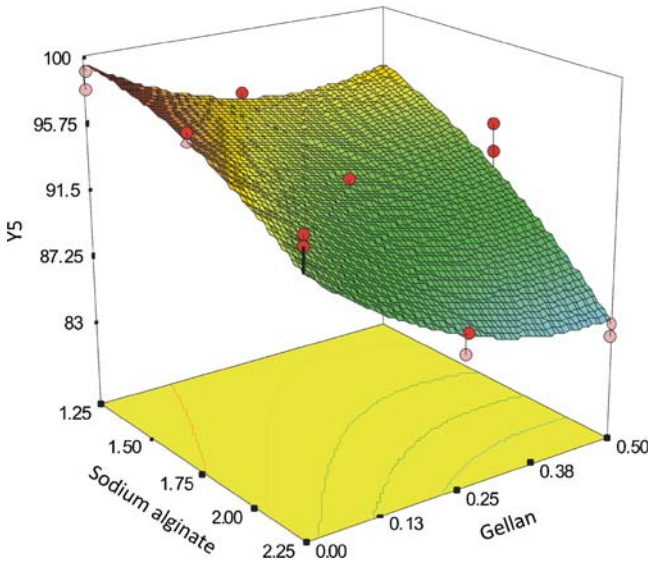

Fig. 5. Surface response plot showing the influence of interaction of sodium alginate and gellan on dependent variable Y_5

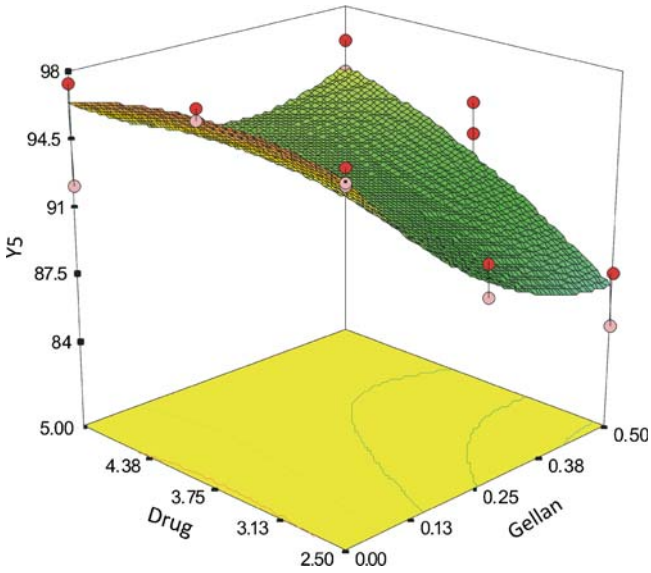

Fig. 7. Surface response plot showing the influence of interaction of drug and gellan on dependent variable Y_5

Fickian mechanism, in the present study, could be attributed to the reason that the formulations during gelation usually imbibe a large amount of dissolution fluid leading to a swollen state of the gel. This might have resulted in the polymeric chain relaxation resulting in non-Fickian mechanism of drug release.

Experimental Designing and Analysis of Variance

The factorial design was carried out using the software DESIGN EXPERT® version 7.0.2.8 (Stat-Ease Inc., Minneapolis, USA). Response surface graphs were used to determine the factor of interaction between the considered variables. A quadratic model was obtained after analyzing data. Values of $p<0.05$ indicate model terms are significant. In this case, X_3, X_2^2 , and X_3^2 are significant model terms. The statistical model comprising incorporated interactive and polynomial terms was utilized to evaluate the response. Once the uncoded values of factor levels were applied and response quadratic model was performed using DESIGN EXPERT® version 7.0.2, equations were obtained. The resulted equations for all five dependent variables—Y_1 (n value), Y_2 (% DE), Y_3 ($X_{30\ min}$), Y_4 ($X_{210\ min}$), and Y_5 ($X_{480\ min}$)—in terms of coded factors are presented below:

$$Y_1 = +0.46 + 0.016X_1 - 4.111E-003X_2 + 0.032X_3 - 0.020X_1X_2 - 0.015X_1X_3 + 3.333E-004X_2X_3 - 7.833E-0X_1^2 + 0.05X_2^2 + 0.07X_3^2$$

$$Y_2 = +70.56 - 2.66X_1 - 2.16X_2 + 1.83X_3 + 0.29X_1X_2 - 0.28X_1X_3 - 1.76X_2X_3 + 0.011X_1^2 + 1.27X_2^2 + 1.20X_3^2$$

$$Y_3 = +31.68 - 4.5X_1 - 4.37X_2 + 2.7X_3 + 2.32X_1X_2 - 0.70X_1X_3 + 0.47X_2X_3 + 0.37X_1^2 + 1.38X_2^2 + 3.8X_3^2$$

$$Y_4 = +64.50 - 6.59X_1 - 4.65X_2 + 3.10X_3 - 1.17X_1X_2 - 0.65X_1X_3 - 0.70X_2X_3 - 0.97X_1^2 + 4.64X_2^2 + 1.27X_3^2$$

$$Y_5 = +92.48 - 4.38X_1 - 2.86X_2 + 1.83X_3 - 1.10X_1X_2 + 0.17X_1X_3 + 2.05X_2X_3 - 1.29X_1^2 + 2.23X_2^2 - 0.96X_3^2$$

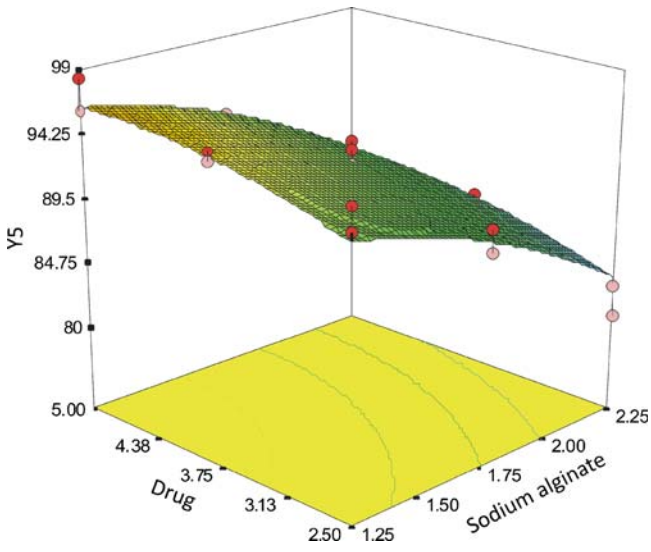

Fig. 6. Surface response plot showing the effect of interaction of sodium alginate and drug on dependent variable Y_5

Table IV. Observed and Predicted Responses and Residual Values for Optimized Formulation (F17) and Market Product

	Formulation (F17)			Market product
Response	Observed	Predicted	Residual	Observed
Y_1	0.49	0.51	−0.02	0.51
Y_2	68.58	69.66	−1.08	68.16
Y_3	25.25	28.69	−3.44	21.29
Y_4	64.05	64.49	−0.435	64.44
Y_5	93.70	91.85	1.85	93.72

Estimation of Quantitative Effects of Factors

A factor was considered to influence the response if the effects are significant ($p<0.05$). A positive value indicates a synergistic effect that favors optimization, while a negative sign represents an antagonistic effect or inverse effect of the factor on the selected response. It is shown in Table III that response Y_1 (release exponent) was significantly influenced by the synergistic effect of drug concentration (X_3), quadratic term of gellan (X_2^2), and metformin (X_3^2) with a respective probability value of 0.0004, 0.0012, and 0.0001. For second response Y_2 (percentage dissolution efficiency), the significant factors were identified as sodium alginate (X_1), gellan (X_2), metformin (X_3), quadratic terms X_2X_3, X_2^2, and X_3^2 with a p value of <0.0001 for all factors except quadratic term X_2^2 ($p=0.0211$) and X_3^2 ($p=0.0282$). Factors X_3, X_2^2 , and X_3^2 had synergistic influence on optimization, while X_1, X_2, and X_1X_2 had antagonistic effect on response Y_2. Significant influence for factor Y_3 was shown by X_1, X_2, X_3, X_1X_2, and X_3^2 with p values of 0.0001 (for first three factors), 0.0012, and 0.0002 for other two factors, respectively. On responses Y_4 and Y_5, factors X_1, X_2, X_3, X_1X_2, and X_2^2 showed significant influence. Among these factors, X_1, X_2, and X_1X_2 have antagonistic effect. Additionally, for response Y_5, factor X_3^2 has showed significant but synergistic effect ($p<0.0001$).

Three-dimensional plots for the measured responses were formed to assess the change of the response surface. Also, the relationship between the dependent and independent variables can be further understood by these plots (Figs. 5, 6, and 7). Based on the model quadratic equations developed, the formulation was optimized on the basis of observed and predicted values for five responses. The optimal factors were determined as sodium alginate 1.75% (*w*/*v*), gellan 0.50% (*w*/*v*), and metformin 3.75% (*w*/*v*). Further, the observed and predicted values of batch F17 correlated significantly with the observed values of marketed metformin tablet (Table IV).

CONCLUSION

In the present investigation, an *in situ* forming formulation for targeting metformin release at upper GIT was developed. Using 3^3 full factorial design, the effect of interaction of three independent variables—sodium alginate, gellan gum, and metformin—on five responses was studied and optimized. Further, the study showed that all three dependent variables had significant effect on the selected responses. The optimized formulation can very well be used as an alternative to the single unit solid dosage form as the former is easy to administer by both geriatric and pediatric patients who constitute a large proportion of diabetic patients in the world.

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