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Research Article

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Development, Optimization, and Anti-diabetic Activity of Gliclazide-Loaded Alginate–Methyl Cellulose Mucoadhesive Microcapsules

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Abstract. The purpose of this work was to develop and optimize gliclazide-loaded alginate–methyl cellulose mucoadhesive microcapsules by ionotropic gelation using central composite design. The effect of formulation parameters like polymer blend ratio and cross-linker ($CaCl_2$) concentration on properties of gliclazide-loaded alginate–methyl cellulose microcapsules like drug encapsulation efficiency and drug release were optimized. The optimized microcapsules were subjected to swelling, mucoadhesive, and *in vivo* studies. The observed responses coincided well with the predicted values from the optimization technique. The optimized microcapsules showed high drug encapsulation efficiency (83.57±2.59% to 85.52±3.07%) with low $T_{50\%}$ (time for 50% drug release, 5.68±0.09 to 5.83±0.11 h). The *in vitro* drug release pattern from optimized microcapsules was found to be controlled-release pattern (zero order) with case II transport release mechanism. Particle sizes of these optimized microcapsules were 0.767± 0.085 to 0.937±0.086 mm. These microcapsules also exhibited good mucoadhesive properties. The *in vivo* studies on alloxan-induced diabetic rats indicated the significant hypoglycemic effect that was observed 12 h after oral administration of optimized mucoadhesive microcapsules. The developed and optimized alginate–methyl cellulose microcapsules are suitable for prolonged systemic absorption of gliclazide to maintain lower blood glucose level and improved patient compliance.

KEY WORDS: alginate–methyl cellulose; anti-diabetic activity; gliclazide; microcapsules; mucoadhesive.

INTRODUCTION

Gliclazide, 1-(3-azabicyclo-[3, 3, 0]-oct-3-yl)-3-(*p*-tolyl sulfonyl) urea, is one of the second generation sulfonylureas used as oral hypoglycemic agent in the treatment of non-insulin-dependent diabetes mellitus (1). Previous reports showed that gliclazide possesses good general tolerability and lower rate of secondary failure (2,3). However, the gliclazide absorption rate from gastrointestinal tract is slow (4). Slower absorption of gliclazide has been suggested which may be due to either its poor dissolution rate owing to its hydrophobic nature or poor permeability across the gastrointestinal membrane (5). Therefore, the incorporation of gliclazide in controlled-release dosage forms such as microcapsules can control its absorption from gastrointestinal tract and thus overcomes variability problems.

Microencapsulation is one of the processes to prolong the drug release and reduce the adverse effects (6). However, the success of microcapsules for controlled drug delivery is limited due to their short residence time at the site of absorption. Therefore, it would be advantageous to have means by providing an intimate contact of the drug delivery systems with the absorbing surface of mucous membranes, *i.e.*, mucoadhesion (7,8). It is mostly achieved by the use of mucoadhesive polymers. The mucoadhesive polymer containing oral drug delivery systems have the capacity to prolong the gastric residence time of drugs at the site of absorption and facilitate intimate contact with underlying absorptive surface to enhance the bioavailability of drugs (9–12).

Over the past few years, pharmaceutical formulators and scientists have shown an increased interest in using alginates as biopolymer in the development of drug delivery systems, due to its hydrogel-forming properties (13,14). These are abundant in nature and found as structural components of brown marine algae (15). Alginate, the monovalent form of alginic acid, belongs to a family of linear co-polymers composed of β-D-mannuronic acid monomers, regions of ∞-L-guluronic acid residues, and regions of interspersed both the residues (16). Alginates undergo ionotropic gelation in aqueous solution in the presence of divalent cations like Ca^{2+},

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Ba^{2+}, *etc.* and trivalent cation like Al^{3+}, due to an ionic interaction between the carboxylic acid groups located on the polymer backbone and these cations (17,18). Alginates have mucoadhesive property, but the cross-linked alginates are usually fragile (19,20). Therefore, to formulate various cross-linked alginate mucoadhesive microcapsules for controlled drug delivery, blending with mucoadhesive polymers is one of the most popular approaches. Again, blending with suitable polymers can improve the drug encapsulation and stability (21), which is found lower in alginate microcapsules, prepared by ionotropic gelation. A few investigations have been carried out to formulate alginate-based mucoadhesive microcapsules or beads for controlled gliclazide delivery. Al-Kassas *et al.* prepared alginate beads of gliclazide by ionotropic gelation (5). In another investigation, various mucoadhesive microcapsules of gliclazide using sodium alginate and mucoadhesive polymers such as sodium carboxymethyl cellulose, carbopol 934 P, and hydroxyl propyl methyl cellulose by ionotropic gelation was formulated by Prajapati *et al.* (22). Nevertheless, it is found that no attempt has been taken to formulate gliclazide-loaded alginate-based microcapsule or bead system using methyl cellulose as a mucoadhesive polymer. Therefore, in the present investigation, an attempt was made to develop and evaluate gliclazide-loaded alginate–methyl cellulose mucoadhesive microcapsules with special reference to anti-diabetic activity.

Designing controlled-release formulations with the minimum number of trials is very crucial for pharmaceutical scientists (23). Central composite design, a response surface design, has been widely used for formulation and process optimization (24). Therefore, the objectives of the present investigation were (a) to evaluate the effect of two process variables like polymer blend ratio and cross-linker concentration on the properties of gliclazide-loaded alginate–methyl cellulose microcapsules like drug encapsulation efficiency and drug release from these new microcapsules; (b) to optimize these process variables, which powerfully influence the properties and performances of gliclazide-loaded alginate–methyl cellulose microcapsules by central composite design; and (c) to evaluate the optimized gliclazide-loaded alginate–methyl cellulose microcapsules *in vitro* and *in vivo*.

MATERIALS AND METHODS

Materials

Gliclazide (Lupin Ltd., India), sodium alginate (CDH Laboratories, India), methyl cellulose (Loba Chemie, India), and calcium chloride (Merck Ltd., India) were used for the present investigation. All other chemicals and reagents used were of analytical grade.

Methods

Preparation of Microcapsules

The microcapsules were prepared by ionotropic gelation technique. Briefly, sodium alginate and methyl cellulose solutions were prepared separately using deionized water and well mixing together. Then, gliclazide was added to the polymeric mixture. The ratio of drug to polymer was maintained 1:1 in all formulations. The final mixture containing gliclazide was homogenized for 10 min at 1,000 rpm using homogenizer (Remi Motors, India), and the resulting mixture was dropped in calcium chloride ($CaCl_2$) solution via 26 gauge needles. After 15 min, the microcapsules were collected by decantation, washed repeatedly using deionized water, and dried at 45°C for 12 h.

Experimental Design

To reduce the number of trials necessary to attain maximum numbers of information on product properties, the screening was performed applying a circumscribed central composite design. The polymer blend ratio (sodium alginate to methyl cellulose, 1:9) and cross-linker concentration ($CaCl_2$, 5:10%, *w/v*) were defined as factors, while drug encapsulation efficiency (DEE; in percent) and time for 50% drug release ($T_{50\%}$, in hours) were used as responses. The process variables (factors) and levels with experimental values are reported in Table I. Design-Expert® Software (V.7.0, Stat-Ease Inc., USA) was used for generation and evaluation of experimental design.

Drug Encapsulation Efficiency (in Percent) Estimation

One hundred milligrams of microcapsules was taken and crushed using pestle and mortar. The crushed powders were placed in 500 ml of phosphate buffer (pH 7.4) and kept for 48 h with occasionally shaking at 37±0.5°C. The polymer debris formed after disintegration of microcapsules was removed by filtering through Whatman® filter paper (no. 40). The drug content in the filtrate was determined quantitatively by UV–VIS spectrophotometer (Shimadzu, Japan) at 226.5 nm wavelength. The DEE (in percent) was calculated using the following formula:

$$\mathrm{DEE}(\%) = \frac{\text{Actual drug content in microcapsules}}{\text{Theoretical drug content in microcapsules}} \times 100 \quad (1)$$

Particle Size Measurement

Average particle size of 100 microcapsules from each batch was measured by optical microscope (Olympus Co., Japan). The ocular micrometer was previously calibrated by stage micrometer.

Table I. Factors and Levels of the Circumscribed Central Composite Design

	Experimental settings	
Normalized levels	SA/MC (X_1)	$CaCl_2$ (%, *w/v*) (X_2)
−1.414	1.00	5.00
−1	2.20	5.70
0	5.00	7.50
1	7.80	9.30
1.414	9.00	10.00

SA/MC sodium alginate-to-methyl cellulose ratio

Table II. Experimental Plan and Observed Response Values from Randomized Run in Central Composite Design

Experimental formulations	Normalized levels of factors		Responses[a]	
	SA/MC (X_1)	$CaCl_2$ (%, *w/v*) (X_2)	DEE (%)	$T_{50\%}$ (h)
F-1	−1	−1	75.55±2.26	4.63±0.05
F-2	−1	1	82.72±2.58	5.57±0.12
F-3	1	−1	63.84±2.04	3.67±0.05
F-4	1	1	68.38±2.12	4.33±0.08
F-5	−1.414	0	83.76±2.66	5.78±0.10
F-6	1.414	0	64.08±2.27	3.83±0.06
F-7	0	−1.414	64.63±2.12	3.97±0.08
F-8	0	1.414	73.60±2.38	4.98±0.08
F-9	0	0	69.68±2.07	4.64±0.08
F-10	0	0	70.39±2.85	4.62±0.09
F-11	0	0	69.95±2.46	4.66±0.08
F-12	0	0	70.07±2.82	4.63±0.10
F-13	0	0	69.31±2.23	4.75±0.07

SA/MC sodium alginate-to-methyl cellulose ratio, *DEE (%)* drug encapsulation efficiency (in percent), $T_{50\%,}$*(h)* time for 50% drug release from microcapsules
[a] Observed response values: mean±SD (n=3)

Morphology Analysis

Microcapsules were gold-coated in an ion sputter (Hitachi E1010, Japan), and morphology was examined by scanning electron microscopy (SEM) (Hitachi S3400, Japan).

In Vitro Drug Release Study

The *in vitro* gliclazide release from microcapsules was tested in 900 ml of phosphate buffer (pH 7.4) using dissolution test apparatus (paddle type) (Campbell Electronics, India) at 37±1°C under 50 rpm speed (25). A sample of microcapsules equivalent to 100 mg gliclazide was used in each test. Five-milliliter aliquot was collected at regular time intervals, and same amount of fresh medium was replaced into dissolution vessel to maintain sink condition throughout the experiment. The collected aliquots were filtered and estimated quantitatively for gliclazide content using UV–VIS spectrophotometer (Shimadzu, Japan) at 226.5 nm wavelength.

Swelling Behavior Evaluation

One hundred milligrams of microcapsules was soaked in phosphate buffer (pH 7.4) and 0.1 N HCl (pH 1.2). The

Table III. Summary of Results of Model Analysis, Lack of Fit, and R^2 Analysis for Measured Responses

Source	DEE (%)				$T_{50\%}$ (h)			
	Sum of squares		*p* value		Sum of squares		*p* value	
Model analysis								
Mean *vs* total	65,957.99				277.48			
Linear *vs* mean	437.35		<0.0001		4.22		<0.0001	
2FI *vs* linear	1.73		0.5551		0.02		0.3570	
Quadratic *vs* 2FI	37.62		0.0002		0.12		0.0227	
Cubic *vs* quadratic	0.46		0.7220		0.04		0.1011	
Residual	3.33				0.03			
Total	64434.49				281.91			
Lack of fit								
Linear	42.48		0.0014		0.20		0.0158	
2FI	40.75		0.0011		0.18		0.0142	
Quadratic	3.13		0.0543		0.05		0.0530	
Cubic	2.67		0.0161		0.01		0.0842	
Pure error	0.67				0.01			
R^2 analysis		Adjusted predicted				Adjusted predicted		
	R^2	R^2	R^2	PRESS	R^2	R^2	R^2	PRESS
Linear	0.9102	0.8922	0.8227	85.21	0.9533	0.9440	0.9031	0.44
2FI	0.9138	0.8851	0.7739	108.62	0.9577	0.9437	0.8813	0.53
Quadratic	0.9921	0.9865	0.9515	23.31	0.9857	0.9754	0.9105	0.40
Cubic	0.9931	0.9834	0.6423	171.90	0.9943	0.9863	0.7881	0.94

DEE (%) drug encapsulation efficiency (in percent), $T_{50\%}$ *(h)* time for 50% drug release from microcapsules, *2FI* two-factor interaction, *PRESS* predicted residual sum of squares

Table IV. Summary of ANOVA for the Response Parameters

Source	Sum of squares	*df*	Mean square	*F* value	*p* value Prob > *F*
For DEE (%)					
Model	476.70	5	95.34	175.87	<0.0001
X_1	363.02	1	363.02	669.64	<0.0001
X_2	74.33	1	74.33	137.12	<0.0001
X_1X_2	1.73	1	1.73	3.19	0.1173
X_1^2	36.63	1	36.63	67.58	<0.0001
X_2^2	0.05	1	0.05	0.09	0.7738
For $T_{50\%}$ (h)					
Model	5.95	5	1.19	4,577.93	<0.0001
X_1	4.18	1	4.18	16,076.90	<0.0001
X_2	1.61	1	1.61	6,191.17	<0.0001
X_1X_2	0.14	1	0.14	526.67	<0.0001
X_1^2	0.02	1	0.02	72.48	<0.0001
X_2^2	0.01	1	0.01	33.33	0.0007

X_1 and X_2 represent the main effects (factors); X_1^2 and X_2^2 are the quadratic effect; X_1X_2 is the interaction effect
DEE (%) drug encapsulation efficiency (in percent), $T_{50\%}$ *(h)* time for 50% drug release from microcapsules

swelled microcapsules were removed at predetermined time interval and weighed after drying their surfaces using tissue paper. Swelling index was determined by using the following formula:

$$\text{Swelling index} = \frac{\text{Weight of microcapsules after swelling} - \text{Dry weight of microcapsules}}{\text{Dry weight of microcapsules}} \times 100 \quad (2)$$

Mucoadhesion Testing

The mucoadhesive properties of microcapsules were evaluated by *in vitro* wash-off method (24). Freshly excised pieces of goat intestinal mucosa (2×2 cm) (collected from slaughterhouse) were mounted on glass slide (7.5×2.5 cm) using thread. About 50 microcapsules were spread onto the wet, ringed tissue specimen, and the prepared slide was hung onto a groove of disintegration test apparatus. The tissue specimen was given a regular up and down movement in a vessel containing 900 ml of phosphate buffer (pH 7.4) and 0.1 N HCl (pH 1.2), separately, at 37±0.5°C. After regular time intervals, the machine was stopped and the number of microcapsules still adhering to the tissue was counted.

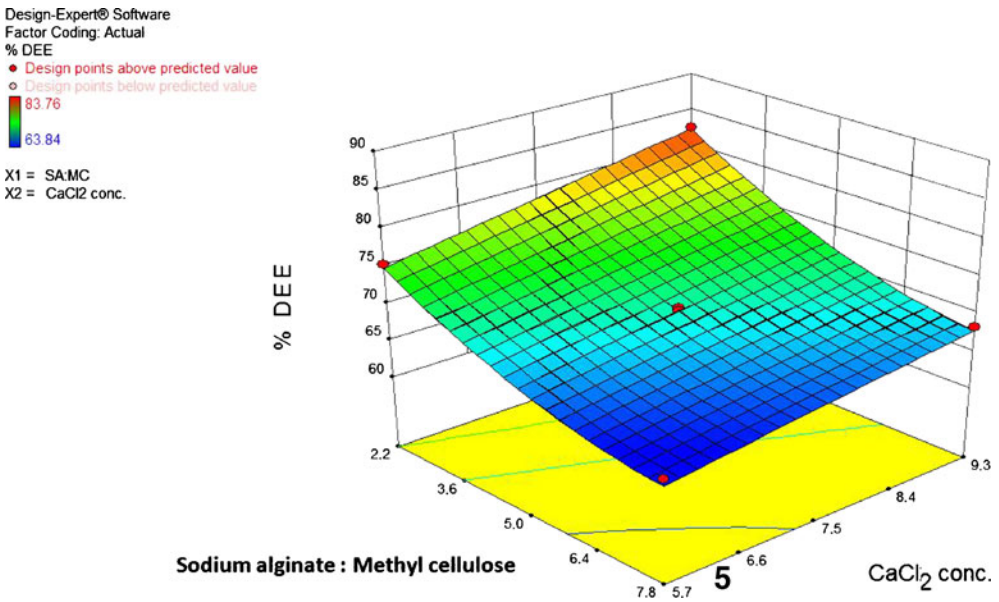

Fig. 1. Effect of main factors on DEE (in percent) presented by response surface plot

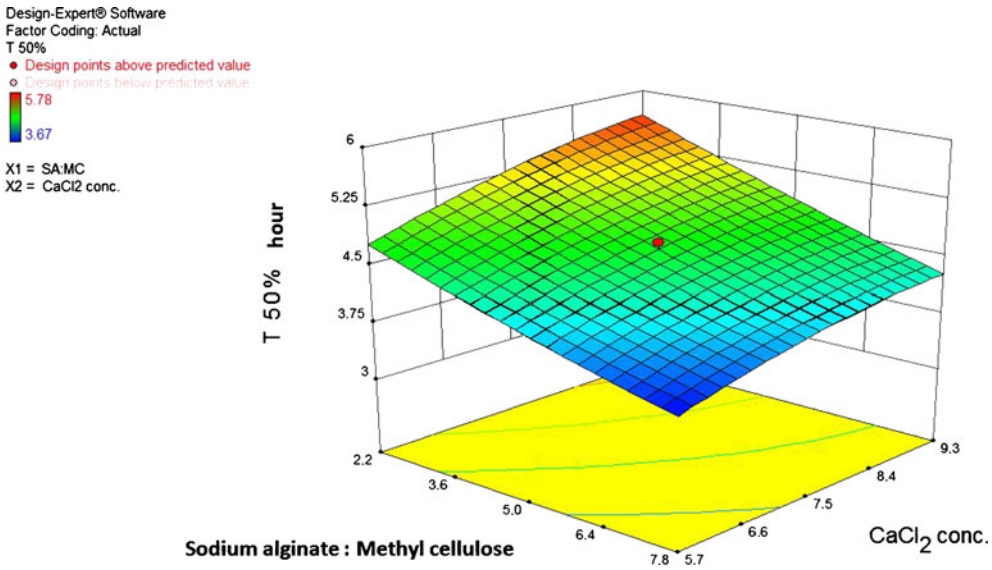

Fig. 2. Effect of main factors on $T_{50\%}$ (in hours) presented by response surface plot

In Vivo Studies

In vivo studies were performed in alloxan-induced diabetic albino rats of either sex (weighing 275–338 g) (22,26). The acclimatized rats were kept fasting for 24 h with water *ad libitum*. All experiments were performed between 8 am to 12 pm to minimize circadian influences.

The animal experimental protocol was approved by the Institutional Animal Ethical Committee and was cleared before starting. The experimental design was subjected to the scrutiny of IFTM University Ethical Committee (reg. no. IFTM/837ac/0159). The animals were handled as per the guidance of the Committee for the Purpose of Control and supervision on Experimental animals (CPCSEA), New Delhi, India. All efforts were made to minimize both the suffering and number of animals used. The rats were made diabetic by intraperitoneal administration of freshly prepared alloxan solution at a dose of 150 mg/kg dissolved in 2 mM citrate buffer (pH 3.0). After 1 week of alloxan administration, alloxanized rats with fasting blood glucose of 300 mg/dl or more were considered diabetic and were employed in the study for 12 h. The alloxan-induced diabetic rats were divided randomly into four groups of three rats each and treated as below.

Group A was administered with pure gliclazide in suspension form. Group B (O-1), C (O-2), and D (O-3) were administered with optimized gliclazide-loaded alginate–methyl cellulose microcapsules, both at a dose equivalent to 2 mg/kg of gliclazide by using oral feeding needle. Blood samples were withdrawn (0.1 ml) from tail tip of each rat at regular time intervals for 12 h under mild ether anesthesia and were analyzed for blood glucose by oxidase peroxidase method using commercial glucose kit. Comparative *in vivo* blood glucose level in alloxan-induced diabetic rats after oral

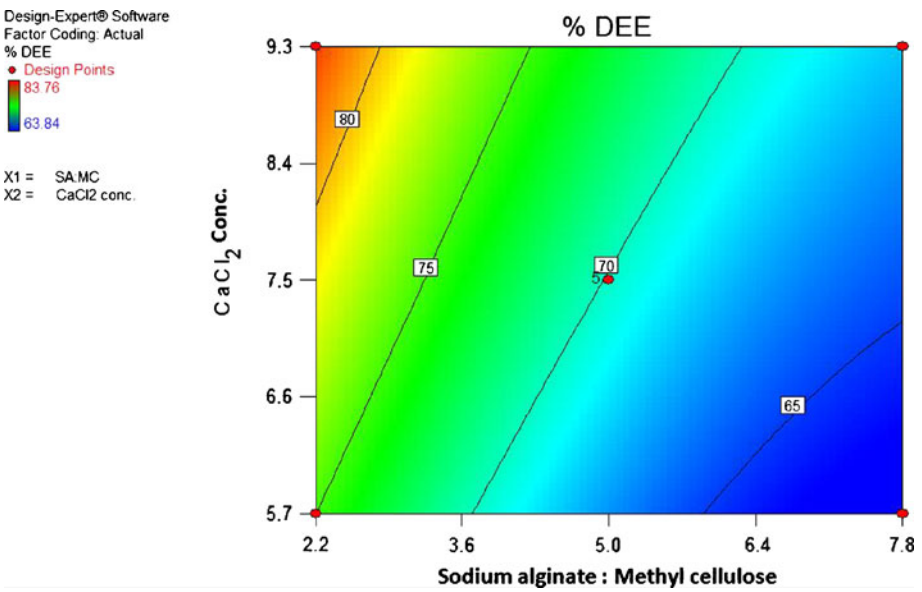

Fig. 3. Effect of main factors on DEE (in percent) presented by contour plot

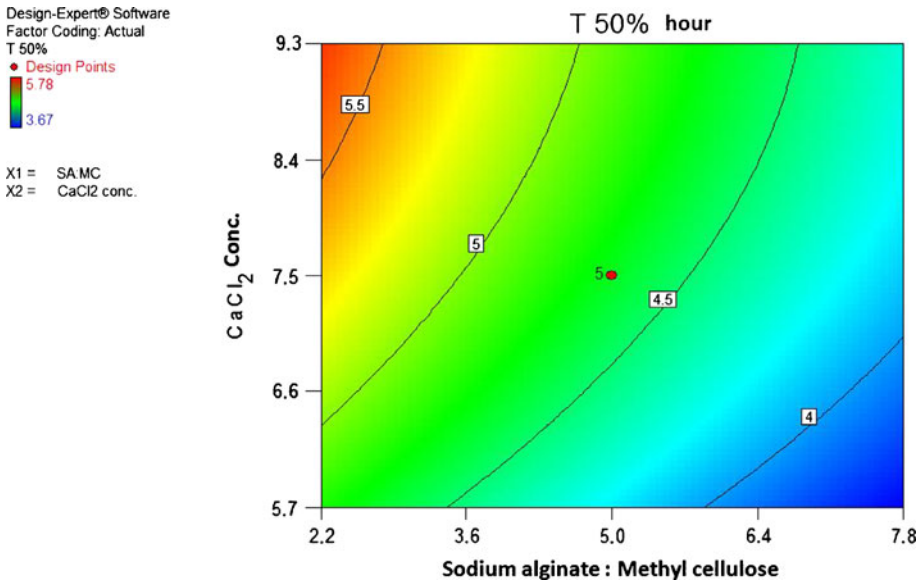

Fig. 4. Effect of main factors on $T_{50\%}$ (in hours) presented by contour plot

administration of pure gliclazide and optimized alginate–methyl cellulose mucoadhesive microcapsules containing gliclazide were evaluated.

Statistical Analysis

For optimization, polynomial equations involving individual factors and interaction factors were selected based on model analysis, lack of fit and R^2 analysis, and predicted residual sum of squares (PRESS) for measured responses. The quadratic mathematical model generated by circumscribed central composite design is in the following (24):

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_1X_2 + b_4{X_1}^2 + b_5{X_2}^2 \qquad (3)$$

where Y is the response; b_0 is the intercept; and b_1, b_2, b_3, b_4, b_5 are regression coefficients. X_1 and X_2 are individual effects; ${X_1}^2$ and ${X_2}^2$ are quadratic effects; X_1X_2 is the interaction effect. One-way ANOVA was applied to estimate the significance of the model ($p<0.05$).

All measured data are expressed as mean±standard deviation (SD). Each measurement was done in triplicate ($n=3$).

RESULTS

Optimization

In the central composite design, total 13 experimental formulations of alginate–methyl cellulose microcapsules containing gliclazide were prepared by ionotropic gelation taking two process variable factors like polymer blend ratio (sodium alginate/methyl cellulose) and cross-linker ($CaCl_2$) concentration (Table I). Overview of the experimental plan and observed response values are presented in Table II. The outcome of model analysis, lack of fit and R^2 analysis, and PRESS value for measured responses are presented in Table III. The model was evaluated statistically applying one-way ANOVA ($p<0.05$), which is shown in Table IV. The model equations were generated to fit the data from the experimental design.

The model equation relating DEE $T_{50\%}$ (in hours) (in percent) as response is shown in Eq. 4:

$$Y_1 = 69.85 - 4.30X_1 + 2.76X_2 + 0.29{X_1}^2 \left(R^2 = 0.9921, p < 0.0001\right) \qquad (4)$$

Table V. Results of Experiments for Confirming Optimization Capability

	Factors		Responses					
			DEE (%)			$T_{50\%}$ (h)		
Code	SA/MC	$CaCl_2$ (%, *w/v*)	Predicted	Observed[a]	Error (%)[b]	Predicted	Observed[a]	Error (%)[b]
O-1	1.00	9.00	87.24	85.52±3.07	1.97	5.96	5.83±0.11	2.18
O-2	1.60	9.50	85.57	83.82±2.77	2.04	5.86	5.78±0.08	1.37
O-3	1.30	8.70	85.23	83.57±2.59	1.95	5.83	5.68±0.09	2.57

SA/MC sodium alginate-to-methyl cellulose ratio, *DEE (%)* drug encapsulation efficiency (in percent), $T_{50\%}$ *(h)* time for 50% drug release from microcapsules

[a] Observed response values: mean±SD ($n=3$)

[b] Error (%)=[Difference between predicted value and observed value/Predicted value]×100

Table VI. Mean Diameter of Alginate–Methyl Cellulose Microcapsules Containing Gliclazide, Measured by Optical Microscopic Method

Formulation codes[a]	Mean diameter[b] (mm)
F-1	0.904±0.097
F-2	0.845±0.084
F-3	0.937±0.086
F-4	0.778±0.068
F-5	0.962±0.092
F-6	0.767±0.085
F-7	0.926±0.087
F-8	0.803±0.078
F-9	0.854±0.080
F-10	0.833±0.091
F-11	0.851±0.068
F-12	0.847±0.068
F-13	0.850±0.092
O-1	0.859±0.084
O-2	0.848±0.079
O-3	0.853±0.090

[a] F-1 to O-3 were alginate–methyl cellulose microcapsules containing gliclazide. Among them, O-1, O-2, and O-3 were optimized formulations
[b] Mean±SD

It can be noted that the coefficient b_3 and b_5 of Eq. 4 had no statistic significance ($p>0.05$) for response Y_1 (DEE, in percent), since the statistic p value of b_3 and b_5 were 0.1173 and 0.7738, respectively.

The model equation relating $T_{50\%}$ (h) as response is shown in Eq. 5:

$$Y_2 = 1.75 - 0.18X_1 + 0.83X_2 - 0.04X_2^2 \left(R^2 = 0.9857, p < 0.0001\right) \quad (5)$$

In Eq. 5, the coefficient b_3 and b_4 had no statistic significance ($p>0.05$) for response Y_2 ($T_{50\%}$, in hours), since the statistic p value of b_3 and b_4 were 0.1849 and 0.1968, respectively.

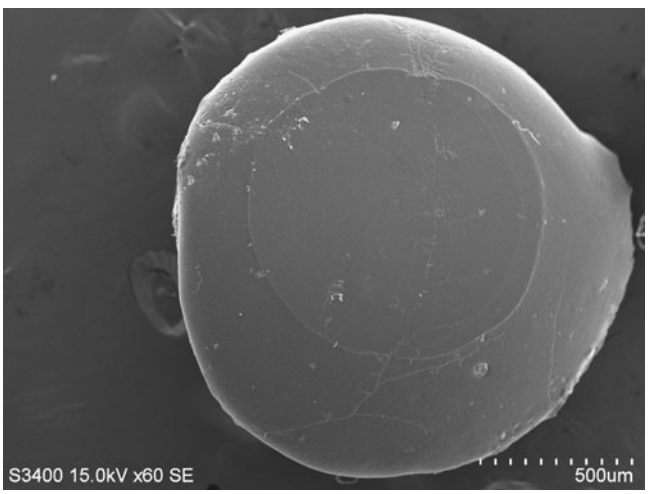

Fig. 5. SEM photograph of gliclazide-loaded alginate–methyl cellulose microcapsules (O-1)

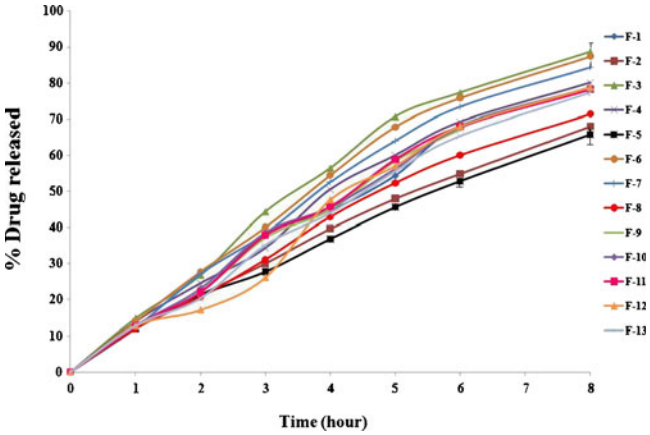

Fig. 6. *In vitro* drug release from alginate–methyl cellulose microcapsules containing gliclazide (F-1 to F-13) (mean±SD, $n=3$)

The three-dimensional response surface plots (Figs. 1 and 2) and corresponding contour plots (Figs. 3 and 4) are presented to reveal the effects of the independent variables on each response.

After generating the polynomial equations relating the responses, alginate–methyl cellulose containing gliclazide were optimized for both responses, Y_1 (DEE, in percent) and Y_2 ($T_{50\%}$, in hours). The desirable ranges of factors were restricted as sodium alginate-to-methyl cellulose ratio within 1:5 and $CaCl_2$ concentration within 5:10% (w/v). In addition, the responses, DEE (in percent) and $T_{50\%}$ (in hours) were restricted to $85\% \leq Y_1 \leq 100\%$ and $5\ \text{h} \leq Y_2 \leq 6\ \text{h}$, respectively. The optimal values of responses were obtained by numerical analysis using the Design-Expert® software (V.7.0, Stat-Ease Inc., USA) based on the criterion of desirability. In order to evaluate the optimization capability of these models generated according to the optimal process variable settings given by the circumscribed central composite design, three formulations of gliclazide-loaded alginate–methyl cellulose microcapsules were selected and formulated. The optimized microcapsules (O-1, O-2, and O-3) were evaluated also for DEE (in percent) and $T_{50\%}$ (in hours). Table V lists the results of experiments with predicted responses by the mathematical model and those observed.

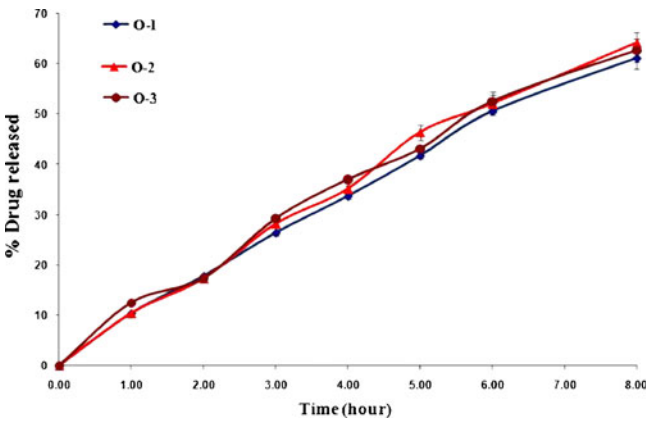

Fig. 7. *In vitro* drug release from optimized alginate–methyl cellulose microcapsules containing gliclazide (O-1 to O-3) (mean±SD, $n=3$)

Table VII. Results of Curve Fitting of the *In Vitro* Gliclazide Release Data from Different Optimized Alginate–Methyl Cellulose Microcapsules

Formulation codes	Correlation coefficient (R^2) values		
	O-1	O-2	O-3
Zero-order model	0.9945	0.9939	0.9924
First-order model	0.9849	0.9816	0.9842
Higuchi model	0.9794	0.9777	0.9792
Korsmeyer–Peppas Model	0.9872	0.9860	0.9761
Diffusion coefficient (n)	0.8697	0.9225	0.8743

Particle Size and Morphology

Particle size of gliclazide-loaded various alginate–methyl cellulose microcapsules was measured by optical microscopic method applied for each formulation. The mean diameters of all these microcapsules are shown in Table VI. The morphological analysis of microcapsules was done by SEM and presented in Fig. 5.

In Vitro Drug Release Studies

The *in vitro* drug release studies were carried out for gliclazide-loaded alginate–methyl cellulose microcapsules in phosphate buffer (pH, 7.4). Various microcapsules (SP-1 to SP-13 and O-1 to O-3) showed prolonged release of gliclazide over 8 h (Figs. 6 and 7). The *in vitro* drug release data of optimized microcapsules were evaluated kinetically using various mathematical models (27–30):

Zero-order kinetics	$F=k_0t$, where F represents the fraction of drug released in time t and k_0 is the zero-order release constant
First-order kinetics	$\ln(1-F)=-k_1t$, where F represents the fraction of drug released in time t and k_1 is the first-order release constant
Higuchi model	$F=k_H\,t½$, where F represents the fraction of drug released in time t and k_H is the Higuchi dissolution constant
Korsmeyer–Peppas model	$F=k_P\,t^n$, where F represents the fraction of drug released in time t, k_P is the rate constant, and n is the diffusion exponent; this indicates the drug release mechanism

The results of the curve fitting into these above-mentioned mathematical models are presented in Table VII.

Swelling Behavior

The swelling behavior of optimized alginate–methylcellulose microcapsules containing gliclazide was evaluated in gastric pH (0.1 N HCl, pH 1.2) and intestinal pH (phosphate buffer, pH 7.4). The swelling index of these microcapsules in both the medium is measured at various time intervals and shown in Table VIII.

Mucoadhesivity

The *in vitro* wash-off test for assessing mucoadhesivity of these optimized alginate–methyl cellulose microcapsules containing gliclazide was performed using goat intestinal mucosa at both gastric pH (0.1 N HCl, pH 1.2) and intestinal pH (phosphate buffer, pH 7.4) for 8 h. The result of *in vitro* wash-off test is presented in Fig. 8.

In Vivo Blood Glucose Evaluation

In vivo efficiencies of optimized mucoadhesive alginate–methyl cellulose microcapsules containing gliclazide (O-1 to O-3) were performed in alloxan-induced diabetic rats and estimated by measuring the blood glucose level. The comparative *in vivo* blood glucose level and the mean percentage reduction in blood glucose level in alloxan-induced diabetic rats after oral administration of pure gliclazide and optimized alginate–methyl cellulose mucoadhesive microcapsules containing gliclazide is presented in Figs. 9 and 10.

DISCUSSION

Gliclazide-loaded alginate–methyl cellulose microcapsules were prepared by ionotropic gelation technique according to the circumscribed central composite design (Table I). The result of experimental run by the central composite design (Table II) noticed that DEE (in percent) was increased with decreasing of sodium alginate-to-methyl cellulose ratio and increasing $CaCl_2$ concentration. This may be due to

Table VIII. Results of the Swelling Behavior of Gliclazide-Loaded Alginate–Methyl Cellulose Microcapsules in pH 1.2 and pH 7.4

Time (h)	Swelling ratio (%)[a]					
	O-1 (pH 1.2)	O-2 (pH 1.2)	O-3 (pH 1.2)	O-1 (pH 7.4)	O-2 (pH 7.4)	O-3 (pH 7.4)
0.5	111.74±1.79	114.63±1.86	110.42±2.04	118.83±2.06	117.98±1.98	113.84±2.33
1	122.67±2.52	108.64±1.44	116.72±2.02	348.49±3.88	350.12±3.73	344.66±3.76
2	122.06±2.26	124.64±2.06	120.06±3.03	716.43±6.06	682.06±6.34	695.75±6.85
3	144.02±2.62	128.24±3.33	137.00±3.17	923.56±6.87	931.34±7.73	924.90±7.22
4	152.36±3.88	154.55±2.05	148.98±3.13	665.33±8.76	660.85±7.05	662.43±7.98
6	156.58±3.05	147.09±2.77	150.06±3.37	190.74±4.45	187.83±4.56	185.07±4.63
8	160.59±3.85	160.06±3.65	158.56±3.56	2.11±0.21	2.02±0.25	2.29±0.15

[a] Mean±SD, $n=3$

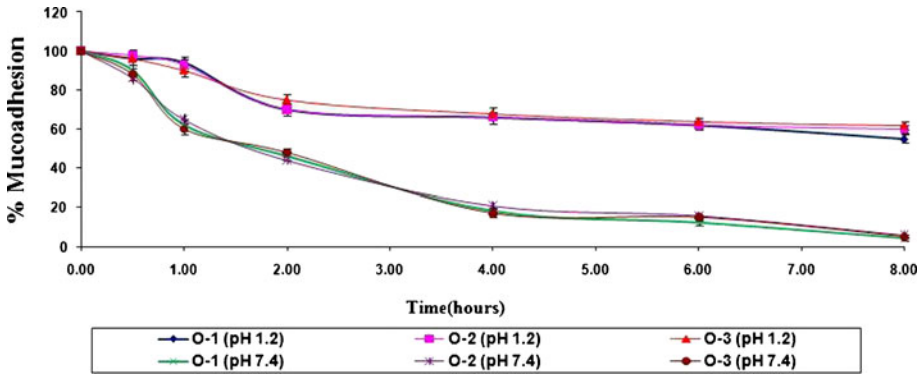

Fig. 8. Results of *in vitro* wash-off test to assess mucoadhesive properties of the optimized alginate–methyl cellulose microcapsules containing gliclazide (mean±SD, $n=3$)

higher degree of cross-linking by $CaCl_2$ and increased viscosity of polymeric solution with methyl cellulose addition. This might have been prevented drug leaching to the cross-linking solution. The microcapsules prepared using lower $CaCl_2$ concentration might have larger pores, due to insufficient cross-linking and resulted lower drug encapsulation (31). However, $T_{50\%}$ (in hours) was decreased with decreasing of sodium alginate-to-methyl cellulose ratio and increasing $CaCl_2$ concentration.

For optimization, the quadratic model was selected based on statistically insignificant lack of fit and smallest values of PRESS for both responses (DEE, in percent and $T_{50\%}$, in hours) (Table III). The smaller the PRESS statistic, the better for the model fitting to data points (32). These models were also evaluated statistically by ANOVA ($p<0.05$) (Table IV), and the result indicated that these models were significant for the responses, studied in this investigation.

The influence of main effects on responses was further elucidated by response surface methodology. The response surface methodology has been widely used for optimization (33,34). The three-dimensional response surface plots (Figs. 1 and 2) and contour plots (Figs. 3 and 4) demonstrate changes in DEE (in percent) and $T_{50\%}$ (in hours) influenced by process variable factors, studied in this investigation.

The optimized microcapsules (O-1, O-2, and O-3) were formulated using selected process variable settings by numerical analysis according to the circumscribed central composite design and evaluated for DEE (in percent) and $T_{50\%}$ (in hours) (Table V). All these optimized microcapsules showed maximum DEE (83.57±2.59% to 85.52±3.07%) with low $T_{50\%}$ (5.68±0.09 to 5.83±0.11 h) with small error values. This reveals that mathematical models obtained by the central composite design were well fitted.

The particle size range of these alginate–methyl cellulose microcapsules were 0.767±0.085 to 0.937±0.086 mm (Table VI). Increasing particle size of microcapsules was found with increasing methyl cellulose incorporation into formulations. This could be attributed due to increase in viscosity of polymer solution with methyl cellulose incorporation in increasing ratio, which increased droplet sizes during addition of polymer solution to cross-linking solution. Again, the particle size of microcapsules was decreased due to shrinkage of polymeric gel by higher degree of cross-linking; when more concentrated $CaCl_2$ solution was used.

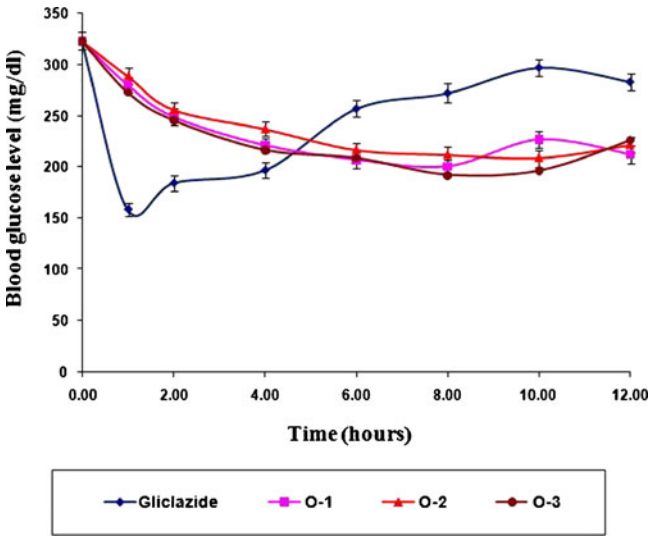

Fig. 9. Comparative *in vivo* blood glucose level in alloxan-induced diabetic rats after oral administration of pure gliclazide and optimized alginate–methyl cellulose mucoadhesive microcapsules containing gliclazide (O-1 to O-3) (mean±SD, $n=3$)

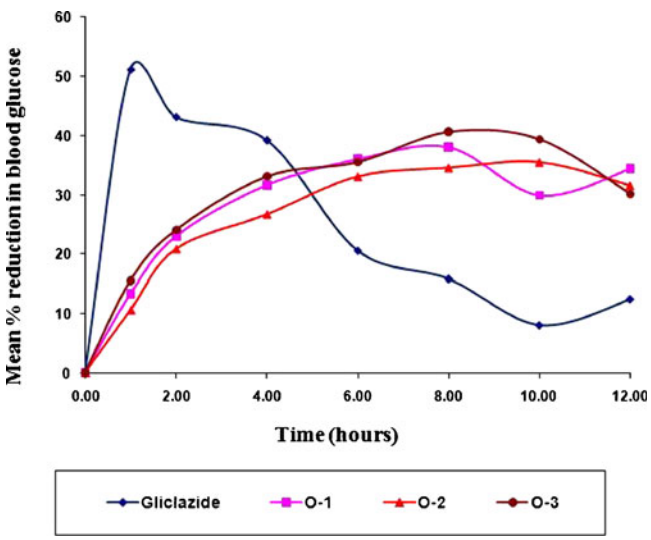

Fig. 10. Comparative *in vivo* mean percentage reductions in blood glucose level in alloxan-induced diabetic rats after oral administration of pure gliclazide and optimized alginate–methyl cellulose mucoadhesive microcapsules containing gliclazide (O-1 to O-3)

Rigid microcapsules were obtained, when polymer (sodium alginate and methyl cellulose)–gliclazide mixture was dropped into $CaCl_2$ solution. The SEM photograph indicated that microcapsules were spherical with rough surfaces and completely covered with the coat polymer (Fig. 5).

Various alginate–methyl cellulose microcapsules (SP-1 to SP-13 and O-1 to O-3) showed prolonged *in vitro* gliclazide release in phosphate buffer (pH, 7.4) over 8 h (Figs. 6 and 7). In case of microcapsules containing higher methyl cellulose amount, the more hydrophilic property of methyl cellulose may bind better with water to form viscous gel structure, which may block the pores on microcapsule surfaces and sustain drug release. The high degree of cross-linking by higher $CaCl_2$ concentration may slower the drug release from highly cross-linked microcapsules. Optimized microcapsules (O-1 to O-3) showed only 61.06±2.02 to 64.12±2.16% of gliclazide release in 8 h (Fig. 6). The gliclazide release from optimized microcapsules was found to follow zero-order kinetics (R^2=0.9924 to 0.9939) over a period of 8 h (Table VII), indicating the controlled drug release from these microcapsules. The Korsmeyer–Peppas model was also employed to distinguish two competing release mechanisms, Fickian diffusional release ($n \leq 0.43$) and case II transport ($n \geq 0.85$) (27). The values of diffusion coefficient (n) ranged 0.8697 to 0.9225 (Table VII), indicating the drug release followed the case II transport mechanism controlled by swelling and relaxation of polymeric matrix.

The swelling index of optimized alginate–methylcellulose microcapsules was lower in acidic pH (1.2) in comparison with that of in alkaline pH (7.4) (Table VIII). Maximum swelling was observed at 2–3 h in alkaline pH; after which, erosion and dissolution took place. The swelling behavior of optimized microcapsules in alkaline pH could be explained by the ion exchange phenomenon between the calcium ion of cross-linked alginate–methyl cellulose microcapsules and the sodium ions present in phosphate buffer, with the influence of calcium sequestrate phosphate ions, which resulted in disaggregation of alginate–methyl cellulose matrix structure leading to matrix erosion and dissolution of swollen microcapsules (35).

In gastric pH, microcapsules adhering to goat intestinal mucosa varied from 55.50±3.26% to 70.67±4.05%, whereas this was from 4.50±0.08% to 6.67±0.15% in intestinal pH (Fig. 7). The rapid wash-off observed at intestinal pH could be due to ionization of carboxyl and other functional groups of polymers, which increased their solubility with reduced adhesive strength (35). The results of wash-off test indicated that these optimized microcapsules had fairly good mucoadhesivity.

A rapid reduction of blood glucose level in alloxan-induced diabetic rats was observed for a period of 2 h after oral administration of pure gliclazide (group A). After that, blood sugar level was recovered toward the normal (Figs. 9 and 10). However, the reductions in blood glucose level of groups treated with optimized microcapsules (groups B, C, and D) were slower than that of the group treated with pure gliclazide (group A). In case of groups treated with optimized microcapsules, the reduction in blood glucose level reached a maximum within 3 to 4 h and was sustained over 12 h after oral administration of optimized mucoadhesive alginate–methyl cellulose microcapsules containing gliclazide, which was almost similar with the previously reported gliclazide-loaded microcapsules by Prajapati *et al.* (22) in alloxan-induced diabetic rat model. A reduction of 25% in blood glucose level is considered a significant hypoglycemic effect (22,25). In the previous report by Prajapati *et al.* (22), it was found that the reduction on blood glucose level was slow and reached maximum reduction within 3 h of oral administration of alginate–methyl cellulose mucoadhesive microcapsules of gliclazide in rat model. So, it can be concluded from the present investigation that the drug release pattern from optimized alginate–methyl cellulose microcapsules was much sustained in comparison to the previously reported mucoadhesive microcapsules containing gliclazide. Therefore, the sustained anti-diabetic effect by optimized microcapsules was observed over a longer period. The above studies also indicated that these mucoadhesive microcapsules swelled slowly in stomach and accordingly adhered to the stomach mucosa allowing more gliclazide to be absorbed by prolonging gastric residence and then subsequently moved to upper intestine, where they swelled more and released drug through the polymeric gel layer, formed at matrices periphery.

CONCLUSION

The optimized alginate–methyl cellulose mucoadhesive microcapsules containing gliclazide by ionotropic gelation was developed based on central composite design. The drug encapsulation efficiency of these optimized microcapsules was found to be maximum (83.57±2.59% to 85.52±3.07%) with a controlled drug release pattern (zero order) and the drug release mechanism followed the case II transport. All of these optimized microcapsules exhibited good mucoadhesive behavior. The *in vivo* study demonstrated that the significant hypoglycemic effect was observed after oral administration of optimized mucoadhesive microcapsules containing gliclazide. Therefore, the developed and optimized alginate–methyl cellulose microcapsules are suitable for prolonged systemic absorption of gliclazide through controlled drug release and mucoadhesive properties after oral administration in the treatment of non-insulin-dependent diabetes mellitus with maintaining lower blood glucose level and improved patient compliance.

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