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FORMULATION BY DESIGN: UNDERSTANDING THE FORMULATION VARIABLES AND OPTIMISATION OF GLIPIZIDE BUOYANT BIOADHESIVE MICROCARRIERS BY CENTRAL COMPOSITE DESIGN

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In this study, a combination of statistical and analytical approach have been used in the formulation development and optimisation guided by "formulation by design" (FbD) for air entrapped microcarriers of alginate of glipizide. Microcarriers of glipizide were prepared by ionotropic-gelation technique, taking concentrations of sodium alginate (SA) and calcium carbonate (CC) as independent variables. The effects of the polymers were evaluated on dependent variables that is drug release (DR), entrapment efficiency (EE), bioadhesive strength (BS), microcarrier size (MS) and total floating time (TFT). Various batches exhibited rough, spherical microcarriers with nominal size variation having sufficient drug EE, very short lag time, while the microcarriers shows the buoyancy over a period of 6–18 hours based on the formulation variables. The DR from the microcarriers was also sustained for more than 10 hours, in 0.1N HCl (pH 1.2). Higuchi's and the first order kinetic modelling indicated a diffusion-controlled release of drug from the microcarriers. The study also demonstrated the influence of SA and CC on drug EE (71.00%–80.30%) and in vitro release (87.10%–99.89%). Higher level of air increased EE but retarded DR rate as compared to a lower level of air containing microcarriers. The concentration of SA and CC had highly significant effect on buoyancy, DR and other dependent variables as level of significance is ≤0.05. The effects studied will pave the way for developing optimised microcarriers.

Keywords: Design of experiment (DoE), Floating microcarriers, Sodium alginate, Ionic-gelation

INTRODUCTION

Formulation by design (FbD) is an essential part of the modern approach to better pharmaceutical quality assurance. In the past, there has been a lack of definition on how product attributes affect the quality of product. Efforts by the Food and Drug Administration to ensure quality is made via tight specifications that are based on observed characteristics of exhibit or clinical trial batches and binding sponsors using a fixed manufacturing process. In this approach, specifications are valued not because they are related to product quality, but because they are able to detect batch to batch differences which may result in potential therapeutic consequences. FbD emphasise the development of pharmaceutical product based on sound scientific principles. It is also helpful in

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achieving the predetermined specifications of a product for ascertaining the predictable quality (Verma *et al.* 2009). So to get the quality product by using FbD a better understanding of the formulation variables and their interactions, if any, should also be well understood. Various design of experiment (DoE) and response surface methodology (RSM) are very helpful in studying formulation variables and their effects on product quality – both individual and combined (Singh *et al.* 2009).

After attaining controlled/sustained release, the main challenge is to develop a novel drug delivery system which may stay for longer duration in the stomach or the upper small intestine for complete release of drug within desired period of time (Hwang, Park and Park 1998; Deshpande *et al.* 1996). Various approaches proposed for increasing gastric residence of delivery systems in the upper gastrointestinal tract (GIT) include floating drug delivery system (FDDS) (Raval *et al.* 2007; Streubel, Siepmann and Bodmeier 2003; Deshpande *et al.* 1997), high-density (Davis, Stockwell and Taylor 1986; Bechgaard and Ladefoged 1978), mucoadhesive (Patel and Chavda 2009; Ponchel and Irache 1998), swelling and expanding (Urquhart and Theeuwes 1984), modified shape and other delayed gastric devices (Chavanpatil *et al.* 2006; Singh and Kim 2000).

Drugs which cannot be well absorbed throughout whole GIT that is the extended-release dosage forms are disadvantageous while extended release stomach retentive dosage forms are desirable (Baumgartner *et al.* 2000). Extended-release stomach retentive dosage forms are also desirable for the drugs with narrow absorption windows, stability and solubility problems in the intestinal or colonic environments, locally acting in the stomach (Streuble, Siepmann and Bodmeier 2003).

Glipizide (Shahala and Fassihi 2006; Parfitt 1999) is an antidiabetic drug (Torotora and Grabowski 2002) that is used for the treatment of the type-II diabetes and with narrow therapeutic index. The recommended adult dose is 5 mg twice daily or 10 mg once daily, due to the low bioavailability and short biological half-life (3.5–4.0 hours) of glipizide following oral administration which favours development of a controlled release formulation. It also leads to reduction in frequency of dosing and drug toxicity which in turn improve patient compliance. Drugs formulated based on gastro retentive drug delivery systems have long residence time in the stomach. In particular, this system helps in improving the oral sustained delivery of drugs which have an absorption window in a particular region of the gastrointestinal tract (Klausner *et al.* 2003). These systems help in continuously releasing the drug before it reaches the absorption window, thus ensuring optimal bioavailability. For glipizide, its absorption occurs in the stomach (Patel *et al.* 2005). In the present investigation buoyant bioadhesive tablets of glipizide were prepared by effervescent approach using hydroxypropyl methyl cellulose K4M (HPMC) and carbopol 934P (CP). The effects of polymers were evaluated on buoyancy, bioadhesive properties and release characteristics of glipizide tablets.

METHODS

Gift sample of glipizide was received from USV Ltd. (Daman, India). Sodium alginate (SA) (low viscosity grade, 250 cp of 2% solution at 25°C), calcium carbonate (CC) were purchased from Loba Chemie Pvt. Ltd. (Mumbai). Calcium chloride dihydrate and hydrochloric acid (35%) were purchased from E Merck India Ltd. (Mumbai). All other chemicals were of analytical grade and were used as such. Glynase XL 10 mg tablets were purchased from Shri Ram Murti Smarak, Institute of Medical Sciences (Uttar Pradesh, India). Double distilled water was used throughout the study.

Design for Formulation of Air Entrapped Microcarriers

First preformulation studies were conducted to find out the optimum stirring rate and cross-linking time, and the same results are used for further study by three square central composite design (CCD) used for formulation of alginate microcarriers, which is the most efficient design in estimating the influence of individual variables (main effects) and their interactions, using minimum experimentation centre point repeated five times i.e. GFB9, GFB10, GFB11, GFB12 and GFB13, and the mean is used in the study. The ratios of drug to polymer (A) and CC concentration (B) were kept as independent variables (Table 1). Preliminary trials were carried out using different concentrations of SA and CC to shortlist the levels required for the optimisation studies. Microcarriers were optimised by finding the effects of these formulation variables on release in 10 hours (Q_{10}), entrapment efficiency (EE), bioadhesive strength (BS), microcarrier size (MS), floating lag time (FLT) and total floating time (TFT).

Table 1: Experimental design with coded and actual values of independent variables.

S. no.	Batch code	Coded level		Actual amount		Glipizide (mg)	Distilled water (mL; to make)
		Factor A (drug:polymer)	Factor B (CC)	Factor A (drug:polymer)	Factor B (CC), %		
1	GFB1	–1	–1	1:5	6	500	100
2	GFB2	+1	–1	1:9	6	500	100
3	GFB3	-1	+1	1:5	10	500	100
4	GFB4	+1	+1	1:9	10	500	100
5	GFB5	–1	0	1:5	8	500	100
6	GFB6	+1	0	1:9	8	500	100
7	GFB7	0	–1	1:7	6	500	100
8	GFB8	0	+1	1:7	10	500	100
9	GFB9	0	0	1:7	8	500	100
10	GFB10	0	0	1:7	8	500	100
11	GFB11	0	0	1:7	8	500	100
12	GFB12	0	0	1:7	8	500	100
13	GFB13	0	0	1:7	8	500	100

Note: GFB – gastro-retentive floating beads

Preparation of Alginate Microcarriers

Microcarriers were prepared by iono-tropic gelation of SA and CC mixture (Han *et al.* 2007). The required amount of SA (w/v) was added in distilled water to make the polymer solution. CC in the required concentration (w/v) was then added to the polymer solution. The mixtures were homogenised at 10000 rpm using a homogeniser (Remi-motors, RQ-122, Vasai, Maharashtra, India) for 5 minutes. Glipizide was then dispersed in the formed suspension according to Table 1. The bubble free drug loaded suspension was extruded, using a 20 gauge syringe needle into 100 mL 0.45 mol L^{-1} of CC solution maintained under gentle agitation (50 rpm) at room temperature. The alginate gel microcarriers were allowed to stand in the solution for 15 minutes before being separated and washed with distilled water. The microcarriers were dried at 40°C and were stored. The time of drying was optimised by weighing the microcarriers repeatedly, until a constant weight was obtained.

Size, Uniformity and Swelling Index of Microcarrier

Microcarriers of the same size and density were prepared by maintaining various factors constant during preparation like viscosity, rate of falling of drops, stirring rate and distance between syringe and gelation media. Variation in any of these parameters during the microcarrier formation process may result in the production of non-homogenous and non-uniform microcarriers, affecting the overall results to an appreciable extent (Furusle *et al.* 2009).

All batches of buoyant microcarriers were visually analysed for shape and colour. External surface of gel microcarriers were studied with a scanning electron microscope. Particle size of the prepared microcarriers was determined using a digital vernier (Mitutoyo South Asia Pvt. Ltd., New Delhi). Twenty dried microcarriers were measured for calculating the mean diameter. The result is expressed as the mean diameter (mm) ± standard deviation. The swelling properties of the microcarriers were carried out using 0.1N HCl. The microcapsules of known weight were placed in 50 mL of 0.1N HCl for 24 hours. At time intervals of 15 minutes for the first 1 hour, 30 minutes for the next 2 hours and 1 hour for the next 4 hours, the microcarriers were removed, excess surface liquid was removed by blotting paper and their weight was recorded (Wagner 1969).

The percentage swelling (S) was determined by the following equation:

S = (weight of swollen microcarrier – weight of dry microcarrier/weight of dry microcarrier) × 100

Surface Morphology

Surface morphology of microcarriers was studied by scanning electron microscopy of microcarriers (Phillips 1500). The microcarriers were previously fixed on a brass stub using double sided adhesive tape and then were made electrically conductive by coating in vacuums, with a thin layer of gold (approximately 300 Å), for 30 seconds and at 30 W. The pictures were taken at an excitation voltage of 15 Kv and at magnification of 65 and 610X.

Estimation of Glipizide

Glipizide content in the floating microcarriers was estimated by a UV spectrophotometer (Shimadzu 1800, Japan) at 274 nm in 0.1N HCl (pH 1.2); the same was used for dilution. The method was validated for linearity, accuracy and precision. The method obeyed Beer's law in the concentration range of 5–50 µg/mL (Indian Pharmacopoeia 2007).

Drug Entrapment Efficiency (EE)

Microcarriers (50 mg) were crushed using a pestle in a glass mortar and the powdered microcarriers were suspended in 10 mL ethanol in a 100 mL volumetric flask. The volume was made up to 0.1N HCl. After sufficient dilution and filtration it was analysed for the drug content. The EE was calculated according to the following formula (Sato, Kawaafaima and Takenchi 2004):

EE (%) = (actual drug content/theoretical drug content) × 100

In Vitro Buoyancy Study

The floating ability was determined using United State Pharmacopeia (USP) dissolution test apparatus type II (Electrolab, TDT-06T, Maharashtra, India). Fifty microcarriers were kept in the vessel and the paddles were rotated at 50 rpm in 500 mL 0.1N HCl (pH 1.2) solution maintained at 37±0.5°C for 18 hours. The floating and the settled portion of microcarriers were collected separately after test. Percentage buoyancy was calculated as the ratio of the number of microcarriers that remained floating to total number. The floating ability of the microcarriers was measured by visual observation and the results of percentage of floating were taken as the average of three determinations. The preparations were considered to have buoyancy, only when all microcarriers floated on the test solution immediately or within a lag time which did not exceed 2 minutes (Elmowafy *et al.* 2009).

In Vitro Glipizide Release Studies

In vitro release studies were carried out on glipizide loaded buoyant microcarriers using USP XXIV dissolution test apparatus-I (Electrolab, TDT-06T, Maharashtra, India). Weighed quantity of microcarriers equivalent to 10 mg of glipizide were introduced into a dissolution basket and the basket was placed in 900 mL simulated gastric fluid (0.1N HCl) maintained at 37±0.5°C and 50 rpm (Prabhakara *et al.* 2008). Aliquots of 5 mL solution were withdrawn at predetermined time intervals and replaced with fresh dissolution medium. The withdrawn samples were analysed for glipizide content spectrophotometrically (Schimadzu 1800, Japan) at 274 nm (Indian Pharmacopoeia 2007). The results of in vitro release data were fitted into various release equations and kinetic models (Ritger and Peppas 1987; Korsmeyer, Gurny and Peppas 1983; Higuchi 1963).

In Vivo Evaluation

The results shows, when pure glipizide suspension was administered in normal healthy Wistar rats, blood glucose levels decreased rapidly and it was observed that maximum reduction of 45.5% was found within 1 hour after oral administration and within 6 hours blood glucose levels rapidly reached its normal level. Meanwhile, when optimised formulation was administered, reduction in blood glucose levels was reached to maximum value within 1 hour after administration and percentage reduction in blood glucose levels was sustained over 10 hours. Reduction in blood glucose levels by 25% is considered as a significant hypoglycaemic effect which is maintained only up to 2 hours after oral administration of the pure glipizide. In the case of glipizide beads with alginate, significant hypoglycaemic effect was maintained for a period of 1 to 10 hours. Thus glipizide floating beads are significantly more effective than immediate release formulation of glipizide in reducing fasting plasma glucose levels.

Optimisation Data Analysis

The response variables which were considered for FbD optimisation included Q_{10}, EE, BS, MS, FLT and TFT. For the studied design, the multiple linear regression analysis (MLRA) method was applied using Design Expert 6.0.6 (Stat-Ease, Minneapolis, USA) software to fit full second-order polynomial equation with added interaction terms to correlate the studied responses with the examined variables:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_1X_2 + b_4X_1^2 + b_5X_2^2 + b_6X_1X_2^2 + b_7X_1^2X_2$$

where b_o is the intercept while b_1–b_7 are constants, and X_1 and X_2 are taken as variables for the given polynomial equation.

The polynomial regression results were demonstrated for the studied responses. Finally, the prognosis of optimum formulation was conducted using a two-stage brute force technique using MS-Excel spreadsheet software (Singh *et al.* 2010; Moghadam *et al.* 2009). First, a feasible space was located and second, an exhaustive grid search was conducted to predict the possible solutions. The region of optimality was ratified using overlay plots, drawn using the Design Expert® software. Four formulations were selected as the confirmatory check-points and these were validated by RSM. The observed and predicted responses were critically compared. Linear correlation plots were constructed for the chosen check point formulations. The residual graphs between predicted and observed responses were also constructed separately and the percent bias (= prediction error) was calculated with respect to the observed responses.

Comparison with Marketed Product

Optimised formulation *vis-a-vis* marketed formulation (Glynase XL once a day tablets) containing 10 mg of glipizide was compared in terms of drug release (DR) profile.

Stability Studies

Optimised formulation was also subjected to accelerated stability studies to determine the changes in release profile and floating characteristics on storage; stability studies were carried out at 40±2°C/75±5% relative humidity (RH) for 3 months (zone II conditions as per ICH Q1 guidelines) in an environment chamber (Jindal S.M. Scientific, New Delhi). The samples were withdrawn periodically and evaluated (Abdelbary *et al.* 2010).

RESULTS

Preliminary trail batches of microcarriers were prepared by using SA, the stirring speed was varied from 50, 75 and 100 rpm and cross linking time also varied (5, 10 and 15 minutes). From these batches, 50 rpm and 15 minutes were the optimum revolution and cross-linking time used for the preparation of floating microcarriers. The cross linking time did not have a significant effect on the percentage of EE.

Size, Uniformity and Swelling Index of Microcarriers

The glipizide floating microcarriers were prepared by simple ionotropic-gelation technique using SA a natural polymer. Polymer concentration (drug:polymer) was an important factor as viscosity of polymer solution effects the size of microcarriers. Three different polymer concentrations of 2.5%, 3.5% and 4.5% (w/v) were selected.

Surface Topography

Surface topography of prepared microcarriers was studied by scanning electron microscopy as shown in Figure 1.

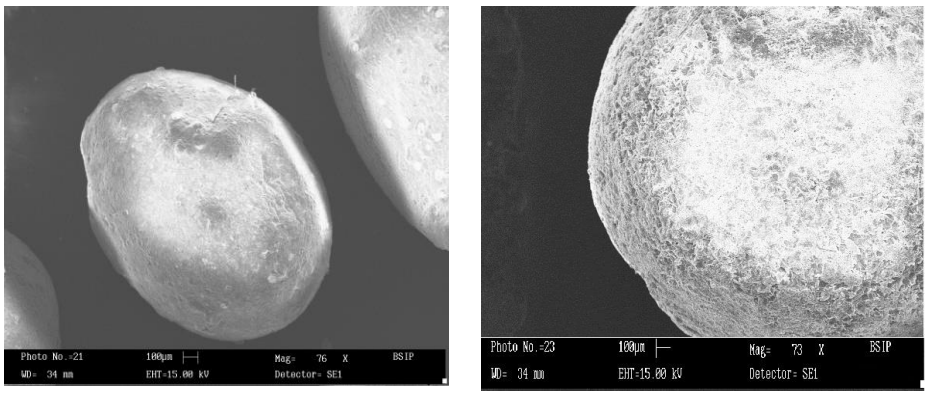


Fig. 1: Scanning electron micrographs of floating microcarriers.

Drug Entrapment Efficiency (EE)

Drug EE is an important variable for assessing the drug loading capacity of microcarriers and their DR profile, thus suggesting the amount of drug availability at site.

In Vitro Buoyancy of Microcarriers

Table 2 shows how the CC concentration (B) loadings affect the buoyancy of the SA microcarriers. All samples with B stayed afloat for >12 hours in an 18-hour test cycle except GFB6 which floated for 2.5 hours. Table 2 also lists the FLT of the drug loaded microcarriers.

In Vitro Glipizide Release Studies

In vitro DR study of glipizide SA microcarriers was carried out in the simulated fasted state, pH 1.2 for a period of 14 hours. In the fasted state, gel microcarriers exhibited a biphasic release profile as an initial rapid DR phase (burst effect) followed by a slower, gradually decreasing DR phase after 1 hour extending up to 14 hours (Table 3 and Fig. 2).

Table 2: Physical evaluation of all the formulation prepared as per the experimental design.

Batch	Size (mm)	Drug EE (%)	Mean density (gm/cm^3)	Force of detachment (Dyne/cm^2)	FLT (second)	TFT (hour)	Shape and colour	Flowability
GFB1	1.52±0.02	71.0±2.4	0.73	3.1±0.2	00	>18	ISOW	FF
GFB2	1.79±0.01	77.5±2.4	0.99	4.5±0.1	07	6.2	ISOWTT	FF
GFB3	1.78±0.01	77.0±2.1	0.66	2.9±0.1	00	>24	ISOW	F
GFB4	1.91±0.01	83.0±4.2	0.86	3.9±0.3	00	>18	ISOWT	F
GFB5	1.59±0.02	75.5±3.2	0.72	3.0±0.2	00	>18	ISOW	FF
GFB6	1.85±0.02	80.3±1.8	0.97	4.2±0.3	05	>18	ISOWT	FF

(*continued on next page*)

Table 2: (*continued*)

Batch	Size (mm)	Drug EE (%)	Mean density (gm/cm^3)	Force of detachment (Dyne/cm^2)	FLT (second)	TFT (hour)	Shape and colour	Flowability
GFB7	1.68±0.01	74.5±1.2	0.87	3.7±0.4	00	>18	IASOW	FF
GFB8	1.86±0.01	79.1±3.2	0.74	3.3±0.2	00	>18	IASOW	F
GFB9	1.76±0.02	76.5±1.5	0.85	3.4±0.6	00	>18	IASOW	FF
GFB10	1.76±0.03	76.6±1.8	0.85	3.4±0.4	00	>18	IASOW	FF
GFB11	1.74±0.02	76.5±1.2	0.84	3.4±0.3	00	>18	IASOW	FF
GFB12	1.75±0.02	75.9±1.7	0.85	3.5±0.3	00	>18	IASOW	FF
GFB13	1.73±0.02	76.7±1.1	0.84	3.4±0.2	00	>18	IASOW	FF

Note: ISOW = irregular spherical off white, ISOWT = irregular spherical off white with tailing, IASOW = irregular almost spherical off white, F = flowing, FF = free flowing

In Vivo Evaluation

The results show, when pure glipizide suspension was administered in normal healthy Wistar rats, blood glucose levels decreased rapidly and it was observed that maximum reduction of 45.5% was found within 1 hour after oral administration and within 6 hours blood glucose levels rapidly reached its normal level (Fig. 3). With the optimised formulation, reduction in blood glucose levels reach the maximum value within 1 hour after administration and percentage reduction in blood glucose levels was sustained over 10 hours. Reduction in blood glucose levels by 25% is considered as a significant hypoglycaemic effect which is maintained only up to 2 hours after oral administration of the pure glipizide. In the case of glipizide beads with alginate, significant hypoglycaemic effect was maintained for a period of 1 to 10 hours. Thus glipizide floating beads are significantly more effective than immediate release formulation of glipizide in reducing fasting plasma glucose levels.

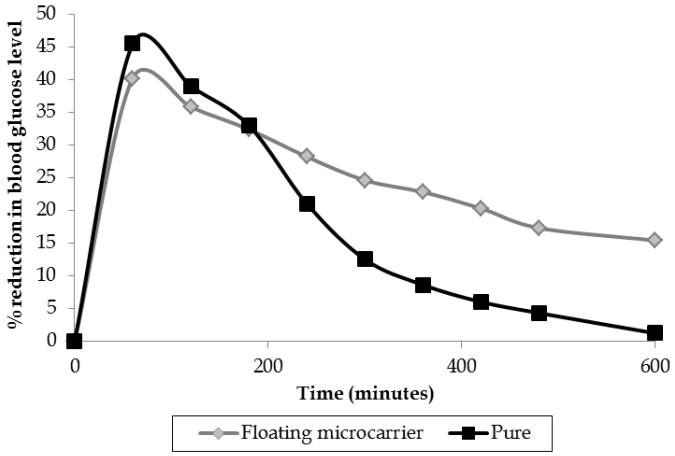


Fig. 2: In intro drug release study of glipizide floating microcarriers.

Table 3: Overall dissolution parameters as per design.

Batch	Q_6	Q_{10}	Q_{14}	Korsmeyer (R^2)	Higuchi (R^2)	First order (R^2)	Zero order (R^2)	n
GFB1	71.34	97.76	98.60	0.9761	0.9917	0.9477	0.8972	0.4596
GFB2	60.80	82.39	87.10	0.9914	0.9951	0.9912	0.9133	0.5061
GFB3	72.33	99.27	99.89	0.9753	0.9913	0.9264	0.9001	0.4681
GFB4	68.43	92.53	96.55	0.9864	0.9932	0.9796	0.9245	0.5154
GFB5	71.66	96.60	98.21	0.9816	0.9941	0.9616	0.8941	0.4632
GFB6	61.31	84.88	88.00	0.9899	0.9945	0.9888	0.9136	0.5011
GFB7	67.76	89.98	95.15	0.9900	0.9947	0.9875	0.9205	0.5170
GFB8	71.00	95.11	97.05	0.9828	0.9945	0.9714	0.8875	0.4568
GFB9	70.00	94.81	96.34	0.9425	0.9722	0.9428	0.8849	0.4470
GFB10	69.17	94.22	96.00	0.9820	0.9939	0.9695	0.8915	0.4584
GFB11	70.00	94.91	97.05	0.9821	0.9938	0.9689	0.8952	0.4638
GFB12	68.35	94.88	96.63	0.9819	0.9934	0.9709	0.8924	0.4598
GFB13	68.67	94.38	96.15	0.9815	0.9936	0.9730	0.8907	0.4568

Note: Q_6 = release in 6 hours, Q_{10} = release in 10 hours, Q_{14} = release in 14 hours

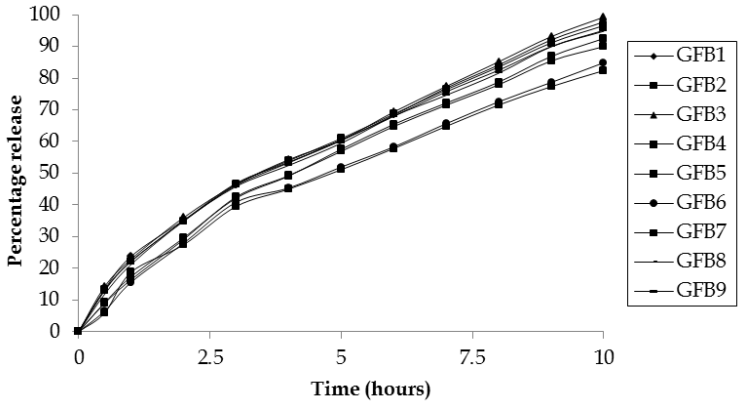


Fig. 3: In vivo evaluation of glipizide floating microcarriers.

Data Analysis and DR Kinetics

The mechanism of DR was investigated by fitting to models representing zero-order, first order, Higuchi's square root of time model and Korsmeyer-Peppas model. First order gave R^2 value of 0.9263–0.9911 describing the DR rate relationship with concentration of drug. The best linearity was found in Higuchi's equation plot, with R^2 between 0.9912–0.9950, indicating the release of drug from matrix as a square root of time dependent process. Results of ANOVA for Response Surface Quadratic Model for various dependent parameters are:

EE = 76.37+2.40*A+4.30*B–0.12*A*B+1.85*A2–1.25*B2–1.43*A2*B+0.72*A*B2

TFT = 18.19+0.000*A+0.000*B+1.38*A*B–0.66*A2–0.66*B2+4.37*A2*B–4.38*A*B2

FLT = 0.28+2.50*A+0.000*B–1.75*A*B+2.03*A2–0.47*B2–1.75*A2*B–0.75*A*B2

MS = 1.75+0.13*A+0.090*B–0.035*A*B–0.024*A2+0.026*B2+5.000E–03*A2*B–0.030*A*B2

DR = 93.99–5.91*A+2.56*B+2.16*A*B–1.74*A2+0.011*B2+0.35*A2*B+0.38*A*B2

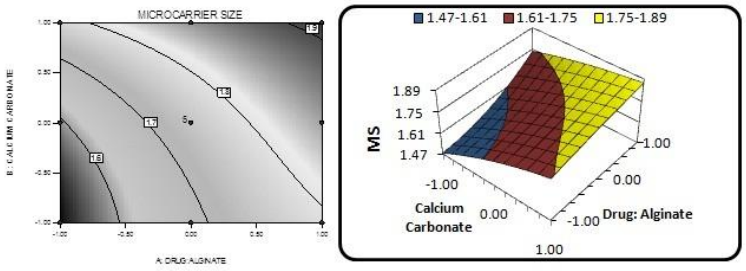
BS = 3.43+0.60*A–0.20*B–0.10*A*B+0.14*A2+0.041*B2+0.000*A2*B+0.000*A*B2

where A is drug to alginate ratio and B represents CC concentration.

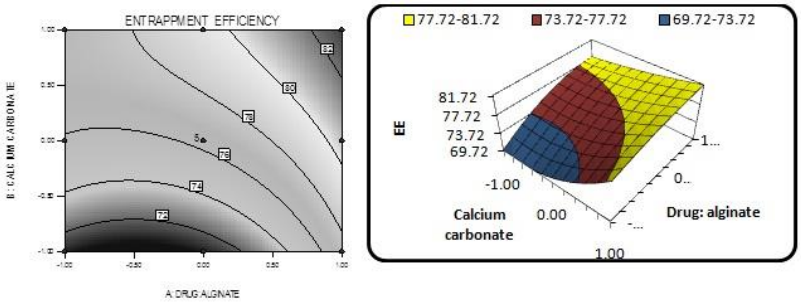
Figure 4 shows response surface and contour plots for MS, EE, BS, TFT and DR.

Design Validation and Selection of Optimum Formulation

By comparing observed and anticipated responses (Table 4), the prediction error varied between –1.380% and 0.448% (mean±SD = 0.27±0.25%). Various linear correlation plots drawn between the predicted and observed responses, forcing the line through the origin, showed high values of regression coefficient (R) (0.9185 to 0.9942) (Figs. 5 and 6), indicating excellent goodness of fit (p<0.005).

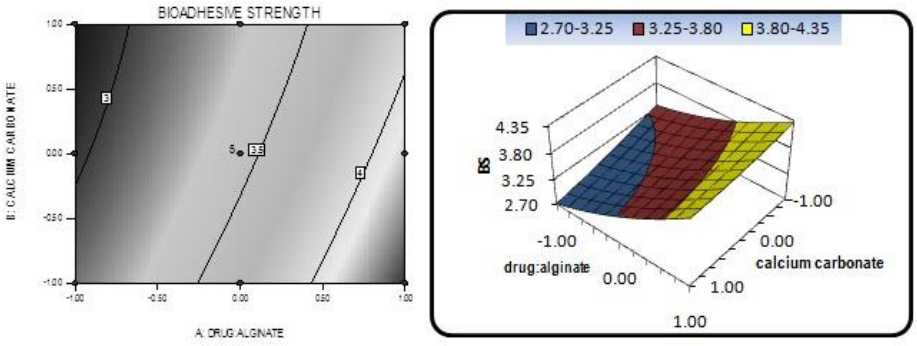


(a)

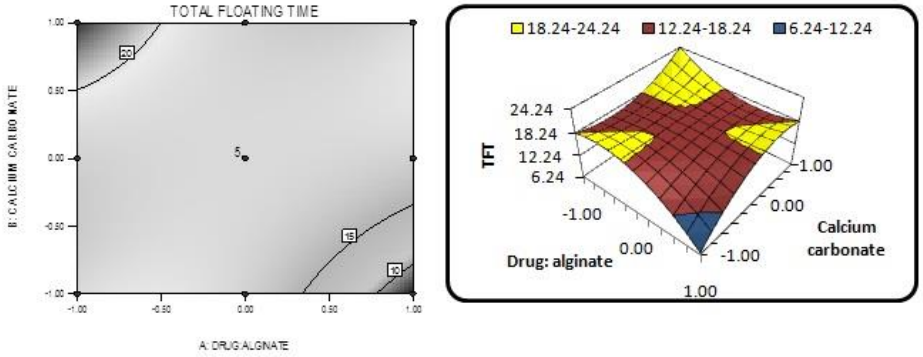


(b)

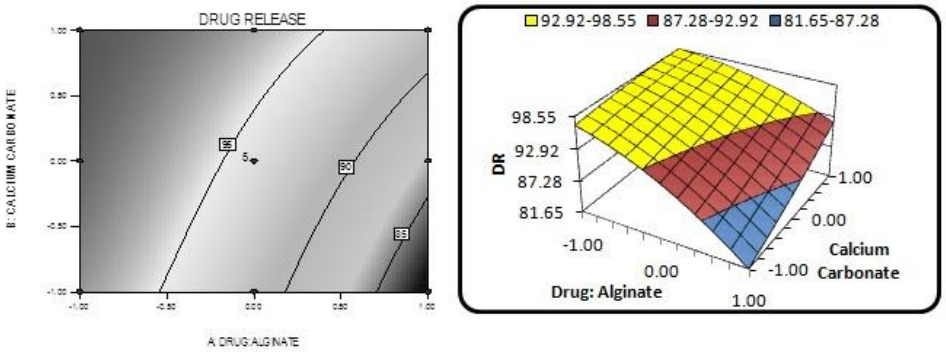
Fig. 4: Response surface and contour plots for (a) microcarrier size, (b) entraptment efficiency, (c) bioadhesive strenght, (d) total floating time and (e) drug release (*continued on next page*).



(c)



(d)



(e)

Fig. 4: (*continued*).

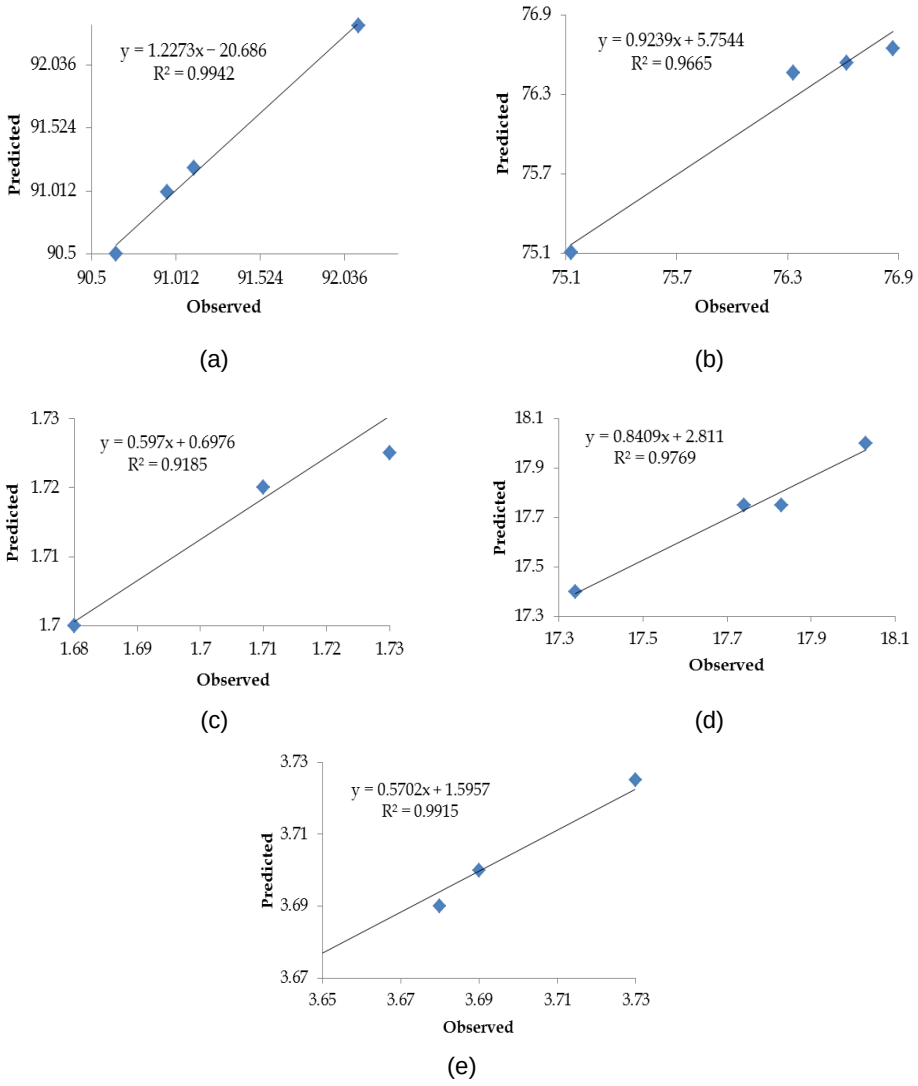


Fig. 5: Regression coefficient between anticipated and experimental response: (a) release in 10 hours, (b) entraptment efficiency, (c) microcarrier size, (d) total floating time and (e) bioadhesive strength (*continued on next page*).

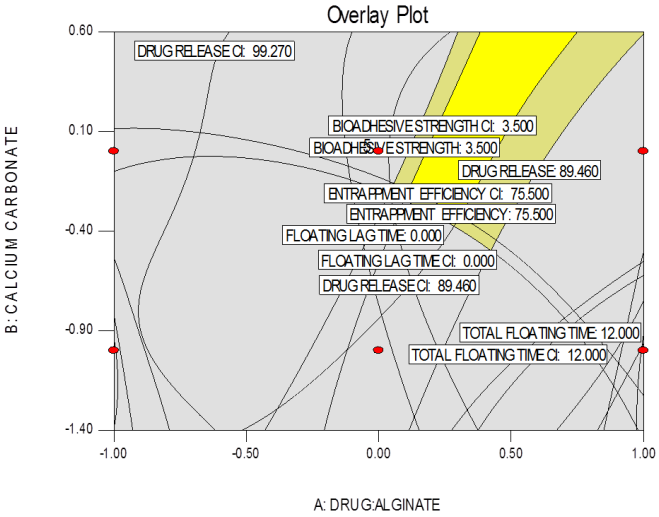


Fig. 6: Overlay plot showing area for optimised product.

Table 4: Validation checkpoint compositions and their results.

Checkpoint batch	A	B	Response variables	Prediction values	Experimental values	Percentage error
VD1	0.40 (1:7.8)	–0.20 (7.60)	Q_{10} (%)	90.65	90.50	0.1654715
			EE (%)	76.87	76.65	0.2861974
			MS (mm)	1.73	1.735	–0.2890173
			TFT (hour)	17.74	17.75	–0.0563697
			BS (d/cm^2)	3.73	3.725	0.1340482
VD2	0.22 (1:7.44)	–0.16 (7.28)	Q_{10} (%)	92.12	92.35	–0.2496743
			EE (%)	76.33	76.46	–0.1703131
			MS (mm)	1.71	1.72	–0.5847953
			TFT (hour)	18.03	18.00	0.1663893
			BS (d/cm^2)	3.60	3.65	–1.3888888
VD3	0.34 (1:7.68)	–0.20 (7.56)	Q_{10} (%)	91.12	91.20	–0.0877963
			EE (%)	76.62	76.54	0.1044113
			MS (mm)	1.73	1.725	0.2890173
			TFT (hour)	17.83	17.75	0.4486819
			BS (d/cm^2)	3.69	3.70	–0.2710027
VD4	0.20 (1:7.4)	–0.60 (7.48)	Q_{10} (%)	90.96	91.00	–0.0439753
			EE (%)	75.13	75.11	0.0266205
			MS (mm)	1.68	1.70	–1.1904761
			TFT (hour)	17.34	17.40	–0.3460207
			BS (d/cm^2)	3.68	3.69	–0.2717391

Note: VD – validation check, A – drug:alginate, B – CC concentration

Comparison of the Optimised Formulation with Marketed Product

Table 5 shows all the DR data of marketed Glynase XL 10 mg extended release glipizide tablet and its comparison with the optimised formulation.

Table 5: Drug release profiles of the marketed brand of glipizide and the optimised formulation.

Formulation	Q_{10} (%)	Q_{14} (%)	n
Glynase XL	82.10	90.14	0.6161
Optimised formulation	90.65	94.26	0.4678

Stability Study of the Optimised Formulation

All the parameters viz., content, TFT, BS and DR remained quite well within the desirable limits, showing negligible and random variation over three months of storage under accelerated conditions. Stability studies were carried out in accordance to ICH guidelines at temperature 40±2°C and relative humidity 75±5%.

DISCUSSION

Concentration of calcium chloride and hardening time had a negative effect on the MS. High calcium chloride concentration and hardening time caused shrinkage of microcarriers and smaller particle are formed because of a high degree of cross linking. This negative effect of calcium chloride concentration and cross linking time was of lesser magnitude they are more due to the morphology of the microcarriers, and the surface became rougher with some very small pores which is in accordance with earlier findings (Moghadam *et al.* 2009).

A concentration of 2.5% [1:5 (drug:polymer)] showed a maximum sphericity and least size. With increase in concentration and hence, the viscosity of SA solutions, microcarriers with larger surface area and less surface porosity were obtained. These release drug slowly. The size of microcarrier is also influenced by the opening through which the SA solution is allowed to pass (which was kept constant). Increased viscosity at a higher concentration of SA resulted in larger particles (1.50–1.92 mm; Table 2). Microcarriers, with more CC concentration, give rougher microcarrier with decreased flowability .

Floating microcarriers of glipizide were irregular-shaped spheres with highly rough surface because of sudden cross linking of SA upon the release of calcium and carbon dioxide from them. The drug-loaded microcarriers were spherical and tailing begins with increasing SA concentrations. Pores or small channels distributed throughout the surface (Fig. 1). Microcarriers were found to be free flowing and of monolithic matrix type. The microcarriers of each batch were uniform in size.

EE ranged from 75% to 87% depending on the composition of the 13 batches of SA microcarriers of glipizide (Table 2). The curing time were kept to 15 minutes since drug is insoluble in water. EE of the microcarriers was found to correlate with the proportion of CC present in microcarriers; with an increase in CC concentration, the drug entrapped increased due to partitioning of the drug in the CC phase. Moreover, an increase in the amount of SA increases EE due to increased space for drug molecules to be retained throughout a larger cross linked network of calcium SA.

The results show that the FLT was decreased for the microcarriers with more air inclusion. At the same time, the concentration of polymer is also governing the FLT, in that

is low polymer concentration resulted in easy floating, and increasing in polymer concentration led to higher FLT. It may be due to the increased density of dried microcarriers and as the volume of microcarriers increases with adsorption of water, its density decreases and begins to float.

GFB1 released 32.29±2.0% glipizide within 1 hour, followed by a tailing off sustained release profile for 14 hours. The initial faster release may be due to drug dissolution from the surface of microcarriers. The DR was found to be slower in formulations with higher air concentration. The slow release of the drug from the microcarriers may be due to the formation of drug-CC dispersion system in the air pockets of the microcarriers. Typically, the drug has to firstly diffuse from the air pockets into the polymeric matrix followed by transportation of drug out of the polymeric matrix into the dissolution medium (Bera *et al.* 2009).

The diffusion exponent (n) value, as calculated from Korsmeyer-Peppas model, for glipizide loaded microcarriers ranged from 0.4470 to 0.5170, showing anomalous (non-Fickian) diffusion involving a combination of swelling, diffusion and/or erosion of matrices in most of batches, except GFB9, as $0.45<n<0.89$ for non-Fickian diffusion.

Figure 3(a) shows a nearly linear ascending pattern for the values of MS, as the content of CC increased; MS also increases with increasing drug:alginate value. Maximum MS is observed at the highest levels of CC and drug:alginate. Contour lines corroborate markedly with more significant influence of CC as compare to drug:alginate on MS.

Figure 3(b) shows a nearly linear ascending pattern for EE, as the content of drug:alginate increased, this EE increases slowly with increasing CC. Maximum EE is observed at the highest levels of drug:alginate and CC. Nonlinear contour lines corroborate markedly showing significant influence of drug:alginate and CC on EE.

Figure 3(c) shows that the BS increases almost linearly with drug:alginate ratio and it decreases very slowly with a CC increase. Maximum BS was found at the highest drug:alginate ratio and the lowest CC combination.

Figure 3(d) portrays the dependency of TFT nonlinearly both on drug:alginate ratio and CC. It increases with CC and decreases with drug:alginate ratio. Contour plot shows that microcarriers were floating for a good time in most of combinations.

According to Figure 3(e), the DR shows a nearly linear descending pattern for the values, as the content of drug:alginate ratio increases or CC decreases, the effect being much more prominent with increasing drug:alginate ratio. Maximum bead size is observable at the lowest levels of drug:alginate ratio and highest CC.

Various linear correlation plots showed high values of R (0.9185 to 0.9942) (Fig. 5), indicating excellent goodness of fit ($p<0.005$). The corresponding residual plots show nearly uniform and random scatter around the mean values of response variables. The optimum formulation was selected by trading off various response variables and adopting the following maximising criteria: MS=1.51–1.90; EE>75%; TFT>15 hours; Q_{10}>90% and BS≥3.5 d/cm^2. Upon comprehensive evaluation of feasibility and grid searches, the formulation (drug:alginate, 1:7.8 and CC, 7.6%) fulfilled the optimal criteria of best regulation of MS=1.51–1.90; EE>75%; TFT>15 hours; Q_{10}>90% and BS≥3.5 d/cm^2, this formulation was taken as the optimised formulation. The same was also confirmed by overlay plot derived through Design Expert^R 8.7.0.1 (Fig. 6).

Dissolution parameter (i.e. DR at 10 hours), obtained during various time points of stability studies carried out in accordance to ICH guidelines at 40±2°C and 75±5% RH, remained almost unaffected during the studies. This suggests the robustness of the optimised formulation with respect to dissolution characteristics.

CONCLUSION

Ease of manufacturing, simplicity and almost complete drug dissolution from the floating drug delivery system are among the various advantages of the developed microcarriers. Results of release studies showed that there are good possibilities of achieving a suitable modulation of microcarrier release rate by varying ratio of polymer. Higuchi's and the first order kinetic modelling indicated a diffusion-controlled release of drug from the microcarriers. FbD optimisation was used as a tool for balancing floatation with release rate. High degree of prognosis obtained using RSM signifies that CCD is quite efficient in studying the formulation variables. Furthermore, the studied responses are helpful in optimising the dosage form, as the formulation with the drug:alginate (1:7.8) and CC (7.6%), was selected as optimum which was also found to be stable. Comparative study with marketed formulation showed comparable and complete release characteristics. Hence, this study could help pave the way for further development in the manufacture of floating controlled release formulations.

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