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Development of Extended Zero-Order Release Gliclazide Tablets by Central Composite Design

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The purpose of this study was to develop an extended release tablet formulation containing gliclazide as a model drug by optimization technique. A central composite design was employed with pH-dependent matrix forming polymers like keltone®-HVCR (X_1) and eudragit®-EPO (X_2) as independent variables. Five dependent variables were considered: hardness, percent drug release after 1 hr, percent drug release after 6 hr, diffusion exponent and time required for 50% of drug release. Response surface methodology and multiple response optimization utilizing a quadratic polynomial equation were used to obtain an optimal formulation. The results indicate that Factor X_1 along its interaction with Factor X_2 was found to be significantly affecting the studied response variables. An optimized formulation, containing 8 mg of keltone®-HVCR and 14.10mg of eudragit®-EPO, provides a sufficient hardness (> 4.5 kg/cm^2) and optimal release properties. The desirability function was used to optimize the response variables, each having a different target and the observed responses were highly agreed with experimental values. The release kinetics of gliclazide from optimized formulation followed zero-order release pattern. The dissolution profiles of optimized formulation before and after stability studies were evaluated by using similarity factor (f_2) and were found to be similar. The results demonstrate the feasibility of the model in the development of extended release dosage form.

Keywords gliclazide; extended release tablets; keltone®-HVCR; eudragit®-EPO; central composite design

INTRODUCTION

Gliclazide is a second-generation sulfonylurea drug, used in the treatment of type-2 diabetes. It is generally well tolerated, is associated with a relatively low incidence of hypoglycemia and has beneficial effects beyond the reduction of serum glucose (Timothy, Frank Daly, John, Kenneth, John, & Leon, 2000). It is also reported that gliclazide reduces plasma cholesterol and triglyceride levels after repeated administration. It is readily and completely absorbed in the gastrointestinal tract and is extensively metabolized in the liver by hydroxylation, *N*-oxidation and oxidation to a number of inactive metabolites (Glowka, Hermann, & Zabel, 1998). The efficacy and safety profiles of gliclazide have been well established and its pharmacological and pharmaceutical properties assessed in numerous studies (Campbell, Laveille, & Nathan, 1991; Palmer & Brogden, 1993). Hence, gliclazide was chosen as a model drug for the present work, which is readily and completely absorbed in the gastrointestinal tract (Delrat, Paraire, & Jochemsen, 2002; Drouin, 2000). Extended release formulations for the antidiabetic drugs are desirable additions to the medical treatment of diabetes as they, in particular, maintain a desirable blood level of a medicament over an extended period, which leads to better management of the disease (Kadhe & Arasan, 2002; Mutalik & Udupa, 2004; Tadeusz, Dobrucki, Piechocki, Resztak, Reh, 2005). Keltone®-HVCR is sodium salt of alginic acid. It is a high-density naturally derived gel-forming polymer. It is free-flowing white to off-white powder, insoluble at pH below 4 and hydrates readily in water (Tønnnesen & Karlsen, 2002). Using alginates alone for the preparation of depot formulations often presents difficulties such as the formulations frequently do not achieve sufficient hardness when tableted, which presents problems during filling (Efentakis & Buckton, 2002; Efentakis & Koutlis, 2001). Conventional alginate-based sustained-release tablets show no plasma level having a pronounced plateau character, despite zero-order in vitro release. These difficulties were avoided, if polyacrylates or polymethacrylates like eudragit-E and eudragit-RS were used along with the alginates (Einig, Stieren, Buehler, & Hollmann, 1993). Based on the above information, in the present study, it was proposed to explore the

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feasibility of incorporating a combination of pH sensitive polymers with opposing solubility behavior in the GI fluids viz., keltone®-HVCR and eudragit®-EPO as the dual matrix forming polymers to extend pH independent gliclazide release. Eudragit®-EPO, a cationic polymer with a dimethylaminoethyl ammonium group is soluble below pH 5 but insoluble and swellable above pH 5 (Moustafine, Zaharov, & Kemenova, 2006).

Design of experiment has been widely used in pharmaceutical field to study the effect of formulation variables and their interaction on dependent (response) variables (Lewis, Mathieu, & Phan-Tan-Luu, 1999; Li, Lin, Daggy, Mirchandani, Chein, 2003; Narendra, Srinath, & Babu, 2006). In the present study, a central composite design (face centered of alpha 1) was proposed for the development of extended release tablet formulations for gliclazide, based on dual combination of matrix forming polymers. The different independent variables include, amount of keltone®-HVCR (X_1) and amount of eudragit®-EPO (X_2). The formulation variables and their ranges were chosen from the knowledge obtained from the preliminary studies. Dependent (response) variables evaluated include hardness, percent drug release after 1 hr, percent drug release after 6 hr, diffusion exponent (*n*) and time taken to release 50% of drug ($T_{50\%}$). The in vitro release studies were performed for the formulated gliclazide tablets and the dissolution data was fitted to curve fitting analysis to obtain the release parameters like $T_{50\%}$ (time taken to release 50% of drug) and diffusion exponent (*n*). All the response variables were fitted to quadratic model and regression analysis was carried out to get a quantitative relationship between the dependent and the analyzed independent variables.

MATERIALS AND METHODS

Chemicals

Gliclazide and polyvinylpyrrolidone (plasdone-K90D) were supplied as gift samples by Microlabs Ltd., Hosur, India. Keltone®-HVCR (viscosity: 400 mPa.s at 20°C; particle morphology: fibrous; particle size: 80 mesh) was supplied as gift sample by International Specialty Products Inc., Bangalore, India. Eudragit®-EPO powder (viscosity: 3–6 mPa.s at 20°C; Particle size: at least 90% was less than 0.315 mm) was supplied as gift sample by Degussa (Rohm Pharma Polymers), Goa, India. Lactose, talc and magnesium stearate were of analytical grade and were obtained from S.D. Fine Chemicals Limited, Mumbai-25, India.

Experimental Design

Central composite design (face centered of alpha 1) is an experimental design technique, by which the factor involved and its relative importance can be assessed was adopted for optimization of extended release tablets of gliclazide (Huang, Tsai, Lee, Chang, Wu, 2005; Mura, Furlanetto, Cirri, Maestrelli, Marras, & Pinzauti,, 2005; Sanchez-Lafuente, Furlanetto, Fernandez-Arevalo, Alvarez-Fuentes, Rabasco, Faucci, Pinzauti, & Mura, 2002; Singh, Sukhwinder, & Ahuja, 2006). According to the model, it contains four full factorial design points, four axial points and three center points. Higher and lower levels of each factor were coded as +1 and −1 respectively, and the mean value as 0. The selected factor levels are summarized in Table 1. The center points were repeated 3 times to estimate the pure experimental uncertainty at the factor levels (Lieberman, Rieger, & Banker, 1988). The two independent formulation variables evaluated include:

TABLE 1
Factor and Level of Studied Variables

Independent Variables		
Coded values	Amount of Keltone®-HVCR (mg) (X_1)	Amount of Eudragit®-EPO (mg) (X_2)
–1	8	8
0	12	12
1	16	16

X_1 = amount of keltone®-HVCR
X_2 = amount of eudragit®-EPO

The response variables tested include:

Y_1 = hardness (kg/cm^2)
Y_2 = percent drug release after 1 hr (%)
Y_3 = percent drug release after 6 hr (%)
Y_4 = diffusion exponent (*n*)
Y_5 = time for 50% of drug release from the delivery system ($T_{50\%}$) (hr)

Preparation of Tablets

The formulations containing 30 mg dose of the drug were prepared at random following the central composite design; Table 2 shows the experimental design. The tablets were prepared by conventional wet granulation technique. Gliclazide, keltone®-HVCR, plasdone-K90D and lactose were blended in a tumbler mixer (Rimek, Karnavati Engineering Ltd., Ahmedabad, India) thoroughly after passing through 60 mesh sieve. The powder blend was wetted with hydro-alcoholic mixture (1:1) in which eudragit®-EPO was dissolved. The wet mass obtained was granulated through 12 mesh sieve and the granules obtained were dried at 50°C ± 5°C in an oven till the moisture content decreased to 2% level (moisture content was determined by using HR73 halogen moisture analyzer, Mettler, Toledo). The dried granules were re-granulated through 22/30 mesh sieve. The oversized granules (retained on 22 mesh sieve) were separated. The undersize granules (passed through

TABLE 2
Central Composite Statistical Design with Observed Responses

FC	(X_1) (mg)	(X_2) (mg)	Hardness (kg/cm^2)*	% Drug Release 1 hr*	% Drug Release 6 hr*	Diffusion Coefficient (n)	$T_{50\%}$ (hr)
F1	8	8	4.03 ± 0.05	10.45 ± 0.458	99.43 ± 1.079	1.15	3.19
F2	16	8	4.00 ± 0.15	10.80 ± 0.029	91.53 ± 1.28	1.07	3.49
F3	8	16	5.03 ± 0.05	13.30 ± 0.087	74.49 ± 0.4	0.88	3.77
F4	16	16	4.50 ± 0.10	8.15 ± 0.229	80.71 ± 0.568	1.25	4.00
F5	8	12	4.43 ± 0.05	11.85 ± 0.999	91.05 ± 0.18	1.11	3.34
F6	16	12	4.10 ± 0.10	9.30 ± 0.087	91.86 ± 0.173	1.21	3.55
F7	12	8	3.83 ± 0.05	12.20 ± 1.076	92.11 ± 0.132	1.02	3.36
F8	12	16	4.67 ± 0.05	12.10 ± 0.926	77.47 ± 1.00	0.96	3.84
F9	12	12	4.17 ± 0.05	10.65 ± 0.15	91.50 ± 0.218	1.07	3.44
F10	12	12	4.17 ± 0.11	11.05 ± 1.004	92.40 ± 0.397	1.07	3.42
F11	12	12	4.20 ± 0.10	11.30 ± 0.328	93.00 ± 0.050	1.08	3.42

All the formulations contained 30 mg of drug; diluent and binder at a fixed concentration of 10% w/w and 5% w/w levels respectively of the total weight of the drug and the polymers. Talc and magnesium stearate were present, each at 2% level of the total weight of the granules obtained.

*All the values are expressed as $M \pm SD$ of three readings.

30 mesh sieve) were mixed with granules (retained on 30 mesh sieve) in a ratio of 1:9 and this granule mixture was lubricated with talc and magnesium stearate. Finally, the lubricated granules were compressed into tablets using 6 mm flat faced punches to a constant pressure of 3 tons in a single punch tablet compression machine (Cadmach, Ahmedabad, India). The total weight of the tablets ranged from 60 mg to 75 mg based on the level of the independent variables used.

Characterization of Granules

Prior to compression, granules were evaluated for their characteristic parameters (Kuksal, Tiwary, Jain, & Jain, 2006). Angle of repose was determined by funnel method; Bulk density (BD) was determined by using a measuring cylinder and Tapped density (TD) was determined by Tap Density Tester (ETD-1020, Electrolab, India) (Banker & Anderson, 1987). Carr's index (CI) was calculated using the following equation,

$$CI = (TD - BD) \times 100 / TD \quad (1)$$

Evaluation of the ER Tablets

Thermal Analysis

Thermal analysis was carried out by differential scanning calorimeter (DSC; Perkin-Elmer, Pyris-1). Randomly selected tablets from each batch of the formulations were crushed together. After pulverization and powder sieving, the mixture was tested by DSC. The thermogram obtained was compared with the thermograms of pure drug and pure polymers. This study was performed to confirm qualitatively the presence of the drug in the formulations and thus to rule out the possible drug-polymers-excipients interaction and the effect of mechanical treatment on the formulations due to the compression process. The instrument was calibrated using indium standards. Accurately weighed samples (10 mg) were hermetically sealed in flat bottom aluminum pans. The scanning was performed at a temperature ranging from 40°C and 240°C at a rate of 10°C/min under an atmosphere of nitrogen.

Characterization of Tablets

The properties of the compressed matrix tablets, such as hardness, friability and weight variation were determined as per USP 24 & NF 19; drug content uniformity was determined as per British Pharmacopoeia, 2001 (BP 2001). Briefly, for each batch, hardness was determined by using Pfizer hardness tester (12061 [USP], Secor, India). Friability was determined using friability testing apparatus (EF-2 friabilator [USP], Electrolab, India). Weight variation of tablets was determined as per official procedure for randomly selected 20 tablets. Uniformity of drug content was determined by using UV double beam spectrophotometer (Pharmaspec UV-1700, Shimadzu, Japan). Ten samples were randomly selected and crushed independently in a mortar; drug equivalent to 30 mg was weighed into 100 mL volumetric flasks containing a small amount of methanol and then diluted with pH-7.4 phosphate buffer solution. After suitable dilutions of 2 mL of the resultant filtrate with the buffer solution, the absorbance of the samples were obtained spectrophotometrically by subtracting the absorbance obtained at 290 nm from the absorbance obtained at 226 nm as per BP 2001 and the drug content was determined from the calibration curve.

In Vitro Dissolution Studies

The drug release profile of the formulated tablets was studied using USP XXIII dissolution apparatus I (TDT-06T, Electrolab, India) in 900 mL of various simulated gastrointestinal fluids viz., in pH 1.2 buffer for the first 2 hr, pH 4.5 for the next 1 hr with 0.1% Tween 80 to increase the wetability of drug and finally in pH 7.4 buffer till 100% of the drug was released as the formulations contained pH sensitive polymers (Chowdary & Kamalakara, 2002; Nath, Venkatesh, & Hiremath, 2000). The temperature of the dissolution medium was maintained at 37 ± 1°C and the stirring speed was set at 50 rpm. Aliquot samples were withdrawn at every 1 hr and after suitable dilutions with pH 7.4 phosphate buffer solution and filtration, the samples were analyzed spectrophotometrically as per BP 2001 and the amount of drug released was determined from the calibration curve. The volume of the sample withdrawn each time was replaced with the same volume of the respective buffer solutions. The studies were carried out in triplicate and mean values plotted verses time with standard error of mean, indicating the reproducibility of the results.

Curve Fitting

Release data were fitted to various mathematical models for describing the release mechanism from tablets; Korsmeyer-Peppas (Eq. 2; Korsmeyer, Doelker, & Peppas, 1983), Zero-order (Eq. 3; Lee, 1984) and Higuchi release models (Eq. 4; Higuchi, 1963).

$$\frac{M_t}{M_\infty} = k_{KP} t^n \tag{2}$$

M_t/M_∞ = fraction of drug released at time 't', k_{KP} is the release rate constant, and n = the release exponent.

$$M_t = M_0 + k_0 t \tag{3}$$

M_t = amount of drug released at time 't', M_0 = concentration of drug in the solution at $t = 0$, k_0 = zero-order release constant.

$$M_t = k_H t^{1/2} \tag{4}$$

M_t = amount of drug released at time '$\sqrt{t}$', k_H = Higuchi release constant.

All curve fitting, simulation and plotting were performed using commercially available SigmaPlot® version 9 (Systat Software, Inc.) and GraphPad PRISM® version 3.02 (GraphPad Software, Inc.) softwares.

Statistical Analysis

The effect of formulation variables on the response variables were statistically evaluated by applying one-way ANOVA at 0.05 level using a commercially available software package Design-Expert® version 6.05 (Stat-Ease, Inc.). The design was evaluated by quadratic model, which bears the form of Eq. 5

$$(Y = b_0 + b_1X_1 + b_2X_2 + b_3X_1^2 + b_4X_2^{\,2} + b_5X_1X_2) \tag{5}$$

where y is the response variable, b_0 the constant and b_1, b_2, b_3 . . . b_5 are the regression coefficients. X_1 and X_2 stand for the main effects; X_1X_2 are the interaction terms, which show how response changes when two factors are simultaneously changed. X_1^2, X_2^2 are quadratic terms of the independent variables to evaluate the nonlinearity.

Scanning Electron Microscopy (SEM)

The surface morphology of the optimized formulation and the longitudinally cut section of the same were analyzed by scanning electron microscope (JEOL-JSM-840A, Japan) after 5 h of dissolution study. The formulation was splutter coated with gold before scanning.

Stability Studies

Stability studies were conducted on the optimized formulation. The optimized formulation was strip packed in Alupoly strip of 0.04 mm thickness and was exposed to 40°C ± 2°C/75% ± 5% RH and 30°C ± 2°C/65% ± 5% RH as per ICH guidelines for 6 months (European Medicines Agency, 2006). Sampling was done at predetermined time intervals of 0, 15, 30, 60, 90, and 180 days. The tablets were evaluated for various physico-chemical parameters viz., appearance, drug content, hardness, and in vitro drug release profiles. To confirm the similarity of drug release profiles before and after stability studies, a model-independent statistical tool for comparison of dissolution profile "*Similarity factor*" (f_2) was used (Costa & Sousa Lobo, 2001).

$$f_2 = 50\ \log\{[1+1/n\Sigma(R_t - T_t)^2]^{-0.5} \times 100\} \tag{6}$$

where, R_t and T_t are percent of drug, which was dissolved at each time point for the reference and test products respectively, n is the number of time points considered. In general, f_2 values higher than 50 (50–100) show similarity of the dissolution profiles.

RESULTS AND DISCUSSIONS

Micromeritics of the Granules

The micromeritic properties were evaluated for all the batches of granules and the results are presented in Table 3. The angle of repose values ranged between 24° (± 1.3) to 28° (± 0.9). The results of angle of repose (< 30°) indicate good flow properties of the granules. The Carr's index is a direct

TABLE 3
Characterization of Tablet Granules

FC	Angle of Repose* (°)	Bulk Density* (g/mL)	Tap Density* (g/mL)	Carr's Index	Moisture Content* (%)
F-1	26 ± 1.4	0.56 ± 0.032	0.69 ± 0.042	18.84	1.4 ± 0.4
F-2	24 ± 1.3	0.63 ± 0.054	0.72 ± 0.084	12.50	1.5 ± 0.3
F-3	28 ± 0.9	0.61 ± 0.015	0.78 ± 0.049	21.79	1.6 ± 0.5
F-4	26 ± 0.9	0.64 ± 0.057	0.76 ± 0.115	15.79	1.6 ± 0.4
F-5	27 ± 0.6	0.60 ± 0.059	0.75 ± 0.069	20.00	1.4 ± 0.1
F-6	25 ± 0.7	0.62 ± 0.083	0.72 ± 0.091	13.89	1.8 ± 0.4
F-7	25 ± 0.9	0.59 ± 0.043	0.71 ± 0.071	16.90	1.9 ± 0.4
F-8	27 ± 0.8	0.57 ± 0.052	0.70 ± 0.078	18.57	1.5 ± 0.2
F-9	26 ± 0.6	0.58 ± 0.042	0.70 ± 0.073	17.14	1.6 ± 0.4
F-10	26 ± 0.9	0.60 ± 0.043	0.73 ± 0.097	17.81	1.9 ± 0.3
F-11	26 ± 0.7	0.59 ± 0.044	0.71 ± 0.070	16.90	1.8 ± 0.2

*All the values are expressed as $M \pm SD$ of three readings.

measure of the propensity of a powder to consolidate when under going vibration, shipping and handling. The results ranged from 12.5 to 20%, which indicates better to fairly good flow properties (Marshall, 1987).

Thermal Analysis

Figure 1 shows the DSC thermograms of the mixture of the crushed tablet formulations, pure gliclazide and pure polymers viz., eudragit®-EPO and keltone®-HVCR. Pure gliclazide had an endothermic peak corresponding to its melting point at 172.283°C. However, except for the negligible shift in the peak corresponding to the drug in crushed formulated tablets mixture to159.771°C and the broadening of the drug peak, no other relevant effects were observed, ruling out any interaction between the drug and all the examined components (Mora, Cirri, & Mura, 2006; Mutalik & Udupa, 2004).

Weight Variation, Assay and Friability of the Formulated Tablets

The average percentage deviation of 20 tablets of each formula was within ± 10%, which provided good weight uniformity as per BP 2001 requirements. In all the formulations, the assay for drug content was found to be uniform among different batches of the dosage form and ranged from 99.97% (± 0.125) to 102.21% (± 0.434) of the theoretical value. Friability is an important parameter related to mechanical resistance of tablets and the results obtained ranged between 0.085 to 0.17%, which indicated that the friability was within the prescribed limits of below 1% (Banker & Anderson, 1987).

Release Profile and Mechanism

Figure 2–4 illustrate the release profiles of the four factorial, four axial and three centre points of the design. It is clear from

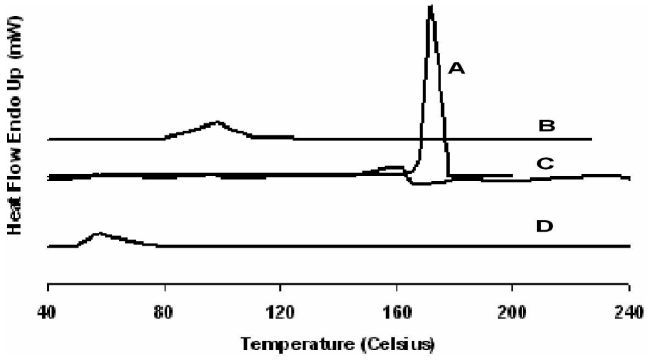

FIGURE 1. DSC thermograms; A = pure gliclazide; B = pure Keltone®-HVCR; C = mixture of crushed formulations; D = pure Eudragit®-EPO.

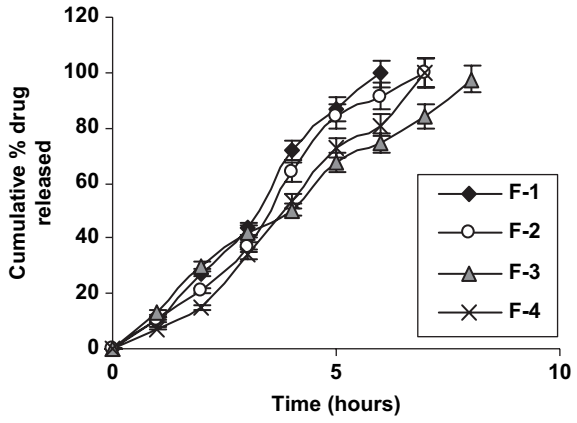

FIGURE 2. Gliclazide release profiles for formulations prepared from four axial points of CCD. All the values are expressed as $M \pm SD$ of three readings.

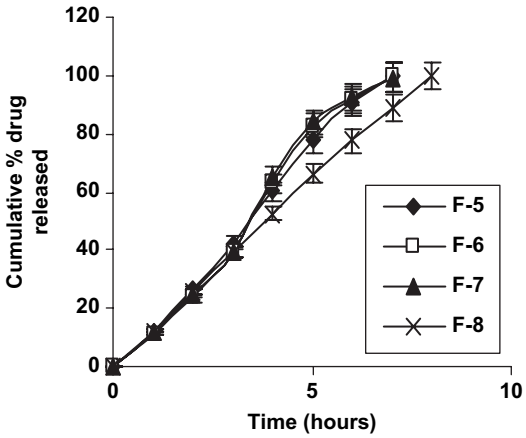

FIGURE 3. Gliclazide release profiles for formulations prepared from four factorial points of CCD. All the values are expressed as $M \pm SD$ of three readings.

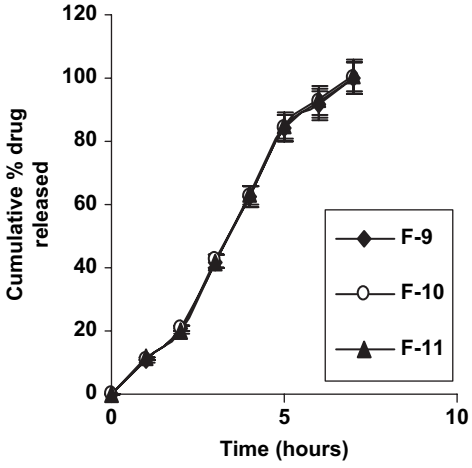

FIGURE 4. Gliclazide release profiles for formulations prepared from three center points of CCD. All the values are expressed as $M \pm SD$ of three readings.

Figures 2–4, that almost all the formulations showed a linear pattern of drug release at least in their initial phase, which indicates the appropriate choice of selected range of formulation variables. On comparison of drug release from the formulations, it was evident that, an increase in both keltone®-HVCR and eudragit®-EPO concentrations in the dosage form decreased the water penetration leading to decreased drug diffusion (as seen in case of formulations F1, F9 or F10 or F11, and F4). From Figure 4, it can be inferred that the release of all three centre points overlaps each other, indicating that the error due to experimental procedure were found to be less in generating a meaningful fitting for the dependent variables. The results of the $T_{50\%}$ values are summarized in Table 2. Formulation F1 showed low $T_{50\%}$ value (3.19 hr) due to rapid release of drug from the delivery system when compared to that of F4 (4 hr). This type of behavior is attributed to high total polymer concentration in the delivery system, which makes the tablet matrix stronger and which in turn leads to slow drug release. However, formulation F1 contains lowest total polymer concentration exhibiting rapid drug release from the delivery system.

In order to understand the complex mechanism of drug release from the tablet, the in vitro release data were fitted to Korsmeyer-Peppas release model (Korsmeyer, 1983) and interpretation of diffusion exponent values (n) enlightens in understanding the release mechanism from the dosage form. For a cylinder, when n is around 0.45, the transport mechanism is by fickian diffusion, and if $0.45 < n < 0.89$, it designates anomalous transport, which is also called as first order release. If $n = 0.89$ or above, it indicates case II or super case II transport, where drug release does not change over time but the release is characterized by polymer relaxation and chain disentanglement, which is often termed as zero-order release (Mohamed, Quadir, & Haider, 2003). The diffusion exponent values thus obtained ranged between 0.88 to 1.25 (Table 2) with limited burst effect, which may be due to controlled initial swelling of hydrophilic keltone®-HVCR and gradual erosion of eudragit®-EPO. These formulations also showed a higher R^2 values for zero-order kinetics, which is illustrated in Table 4, indicating that in addition to fickian diffusion, polymer relaxation also played an important role in controlling the drug release (Skpoug, Borin, Fleishaker, Cooper, 1991).

Experimental Design, Regression Analysis and Model Building

Gliclazide extended release tablet dosage form was formulated following two-factor three-level central composite design (face centered of alpha 1) as the response surface methodology. Totally 11 experiments were implanted and the total tablet weight of which ranged from 60 mg to 75 mg (Table 2). The values of independent variables and the experimental runs with observed responses are presented in Tables 1 and 2, respectively. Eight batches showed less than 12% of drug release at 1 hr (Y_2) and the range of Y_2 of all the batches was 8.15%–13.30%. The ranges of the other responses Y_1 (hardness of tablets [kg/cm^2]), Y_3–Y_5 (% drug release at 6 hr, diffusion coefficient and $T_{50\%}$) were 3.83–5.03 kg/cm^2, 74.49%–99.43%, 0.88–1.25 and 3.19–4 hr respectively. Only statistically significant ($P < 0.05$) coefficients are included in the polynomial equations. A positive sign represents a synergistic effect, while negative sign indicates an antagonistic effect.

Effect of Independent Variables on the Studied Responses

The regression coefficients for each term in the regression model are summarized in Table 5. Table 6 describes the model parameters affecting the response variables hardness and release parameters. In the case of hardness (Y_1), factor X_2 was

TABLE 4
Results of Curve Fitting Analysis

Formulation Code	Korsmeyer-Peppas K_{KP} (hr^{-n})*	R^2	Zero-Order K_0 (% hr^{-1})*	R^2	Higuchi K_H (% $hr^{-1/2}$)*	R^2
F1	13.16 ± 1.89	0.9897	16.63 ± 0.53	0.9829	33.61 ± 3.56	0.8195
F2	12.78 ± 2.64	0.9755	14.93 ± 0.54	0.9727	32.48 ± 3.15	0.8176
F3	15.66 ± 0.93	0.9964	12.52 ± 0.24	0.9895	29.47 ± 1.66	0.9076
F4	8.62 ± 1.37	0.9895	13.56 ± 0.54	0.9704	29.21 ± 3.30	0.7781
F5	14.06 ± 1.29	0.9943	14.74 ± 0.24	0.9940	32.24 ± 2.64	0.8572
F6	13.53 ± 2.29	0.9821	14.92 ± 0.45	0.9809	32.55 ± 2.94	0.8349
F7	14.51 ± 2.52	0.9797	15.06 ± 0.46	0.9796	32.93 ± 2.84	0.8446
F8	13.74 ± 0.47	0.9990	12.79 ± 0.10	0.9984	29.93 ± 2.00	0.8812
F9	13.38 ± 2.47	0.9795	14.97 ± 0.49	0.9779	32.64 ± 3.03	0.8290
F10	13.48 ± 2.49	0.9792	15.05 ± 0.49	0.9777	32.8 ± 3.03	0.8292
F11	13.33 ± 2.48	0.9792	15.13 ± 0.50	0.9773	32.97 ± 3.09	0.8262

*All the values are expressed as $M \pm SD$ of three readings.

TABLE 5
Regression Coefficients for the Response Variables

$Y_1 = 4.17 - 0.15X_1 + 0.39\ X_2 + 0.11\ X_1^2 - 0.13X_1X_2$

$Y_2 = 11.22 - 1.23X_1 - 0.98\ X_1^2 - 1.37X_1X_2$

$Y_3 = 91.75 - 8.40X_2 - 6.14\ X_2^2 + 3.53X_1X_2$

$Y_4 = +1.07 + 0.065\ X_1 - 0.027\ X_2 + 0.093\ X_1^2 - 0.076\ X_2^2 + 0.11\ X_1X_2$

$Y_5 = 3.43 + 0.12\ X_1 + 0.26\ X_2 + 0.17\ X_2^2$

found to be highly significant (< 0.0001) i.e., as the concentration of eudragit®-EPO increased, the hardness of the tablet also increased. But, opposite effect was observed by increasing the amount of keltone®-HVCR and the effect was found to be minimal. Further, the interaction between factors X_1 and X_2 can be elucidated by using response surface plot as illustrated in Figure 5. High level of X_2 showed high value of hardness at all the levels of X_1. As for example, a tablet hardness of $\geq$ 4.5 kg/cm^2 can be obtained if the amount of eudragit®-EPO is kept at higher value irrespective of the amount of keltone®-HVCR in the tablet.

The percent drug release after 1 hr (Y_2) was predominantly dominated by the factor X_1. As the amount of keltone®-HVCR increased, it caused an increase in density of the swollen hydrogel network and decreased the drug diffusion; keltone®-HVCR is a derivative of sodium alginate, which at low pH hydration forms a high viscosity alginic acid gel due to internal bonding (Tapia-Albarran & Villafuerte-Robles, 2004). The interaction between factors X_1 and X_2 can be elucidated by using response surface plot as illustrated in Figure 6. A negligible effect on drug release was observed by increasing X_1 from −1 to +1 level and keeping X_2 at lower level. But the same percent drug release drastically decreased from 13.30 to 8.15% when the amount of X_1 was increased from −1 to +1 level by keeping X_2 at higher level. This may be due to formation of interpolyelectrolyte complexes between eudragit and sodium alginate, which provides higher resistance for the eudragit layer to erode (Moustafine, Kemenova, & Mooter, 2005).

The model terms for the percent drug release after 6 hr (Y_3) was found to be significant with an F value of 215.798. High R^2 value of 0.9831 indicates the adequate fitting to quadratic model. In this case, the percent drug release after 6 h totally depended upon the amount of eudragit®-EPO i.e., as the amount of eudragit®-EPO increased the drug release decreased, which is due to swellable behavior of eudragit®-EPO at pH above 5 by forming a gel matrix. The interaction between factors X_1 and X_2 can be elucidated by using response surface plot as illustrated in Figure 7. If X_1 was kept at low level and X_2 was increased from −1 to +1 level, the percent drug release after 6 hr decreased from 99.43 to 74.49%. Similar type of behavior was also observed when X_2 was increased from −1 to +1 level by keeping X_1 at higher level, i.e., the percent drug release after 6 hr decreased from 91.53 to 80.71%. The probable explanation for this may be due to increased density of swollen hydrogel network of eudragit®-EPO. As discussed earlier, the swellable property of eudragit®-EPO at pH above 5 which forms an alkali gel, leads to a stronger tablet matrix and thus contributes more hindrance for drug diffusion and consequently decreases the drug release at higher pH (Rao, Engh, & Qiu, 2003).

In case of diffusion exponent (Y_4), the factor X_1, X_2 and its quadratic effect were found to be significant. As the concentration of keltone®-HVCR increased, the diffusion exponent value

TABLE 6
Model Parameters for the Studied Response Variables

Source	*df*	*SS*	*MS*	*F* Value	*p*
Hardness (kg/cm^2)				R^2 = 0.9949	
X_1	1	0.13	0.13	109.22	0.0001
X_2	1	0.91	0.91	754.98	< 0.0001
X_1^2	1	0.033	0.033	27.21	0.0034
X_2^2	1	0.025	0.025	20.52	0.0062
X_1X_2	1	0.063	0.063	51.71	0.0008
Drug release at 1 hr (%)				R^2 = 0.9539	
X_1	1	9.00375	9.00375	48.29565	0.0009
X_1^2	1	2.421268	2.421268	12.98755	0.0155
X_1X_2	1	7.5625	7.5625	40.56486	0.0014
Drug release at 6 hr (%)				R^2 = 0.9831	
X_2	1	423.4953	423.4953	215.798	< 0.0001
X_2^2	1	95.63373	95.63373	48.7315	0.0009
X_1X_2	1	49.88283	49.88283	25.41849	0.0040
Diffusion exponent (n)				R^2 = 0.9927	
X_1	1	0.025558	0.025558	159.3549	< 0.0001
X_2	1	0.004444	0.004444	27.71102	0.0033
X_1^2	1	0.021998	0.021998	137.154	< 0.0001
X_2^2	1	0.014504	0.014504	90.43214	0.0002
X_1X_2	1	0.050986	0.050986	317.8917	< 0.0001
$T_{50\%}$ (hr)				R^2 = 0.9956	
X_1	1	0.093483	0.093483	183.1465	< 0.0001
X_2	1	0.414223	0.414223	811.5227	< 0.0001
X_2^2	1	0.071851	0.071851	140.767	< 0.0001

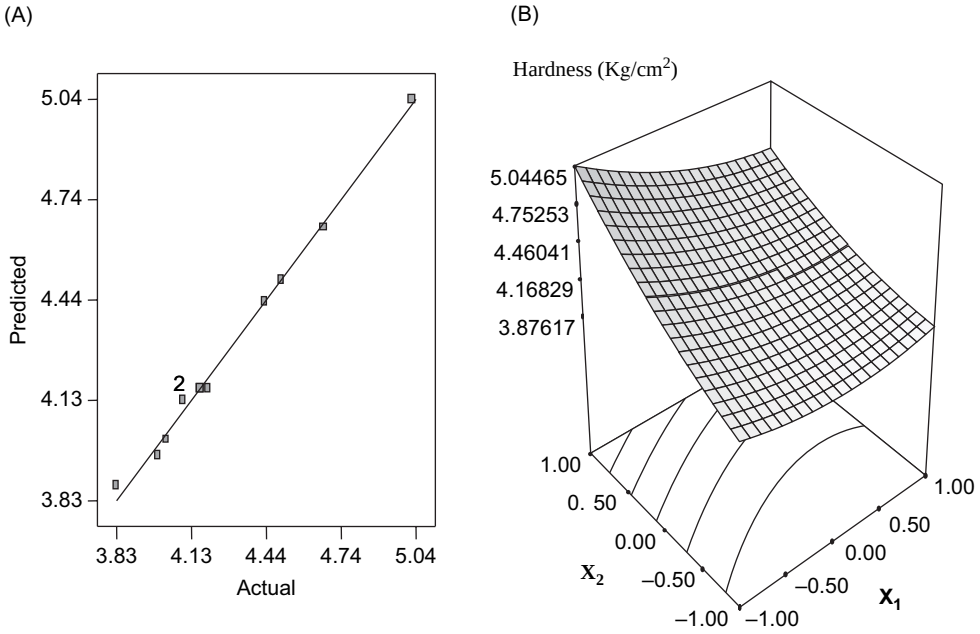

FIGURE 5. (A) Correlation between actual and predicted values. (B) Response surface plot showing the effect of X_1 and X_2 on the response hardness (Y_1).

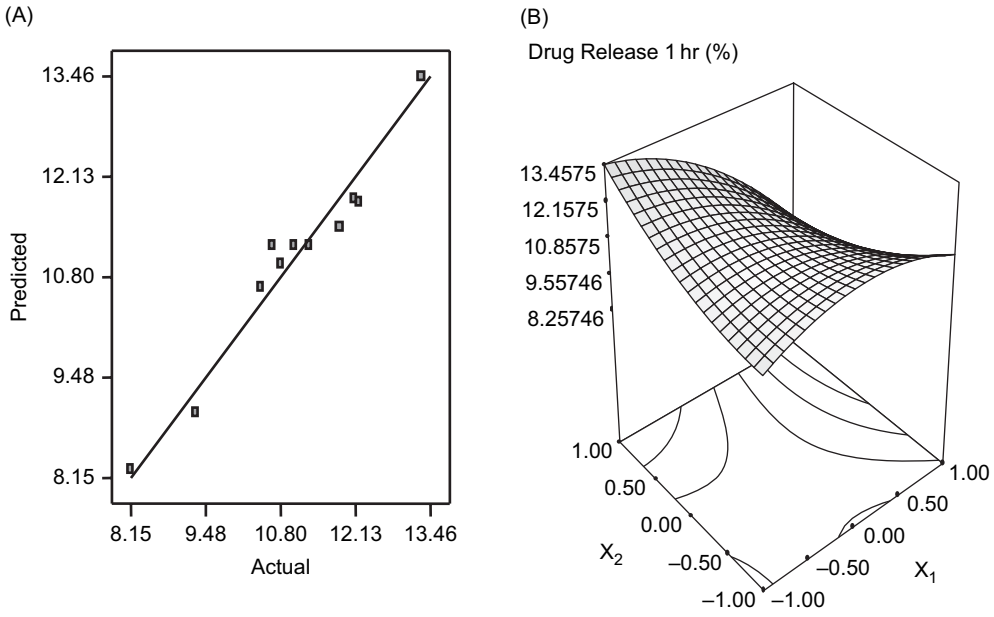

FIGURE 6. (A) Correlation between actual and predicted values. (B) Response surface plot showing the effect of X_1 and X_2 on the response drug release 1 hr (Y_2).

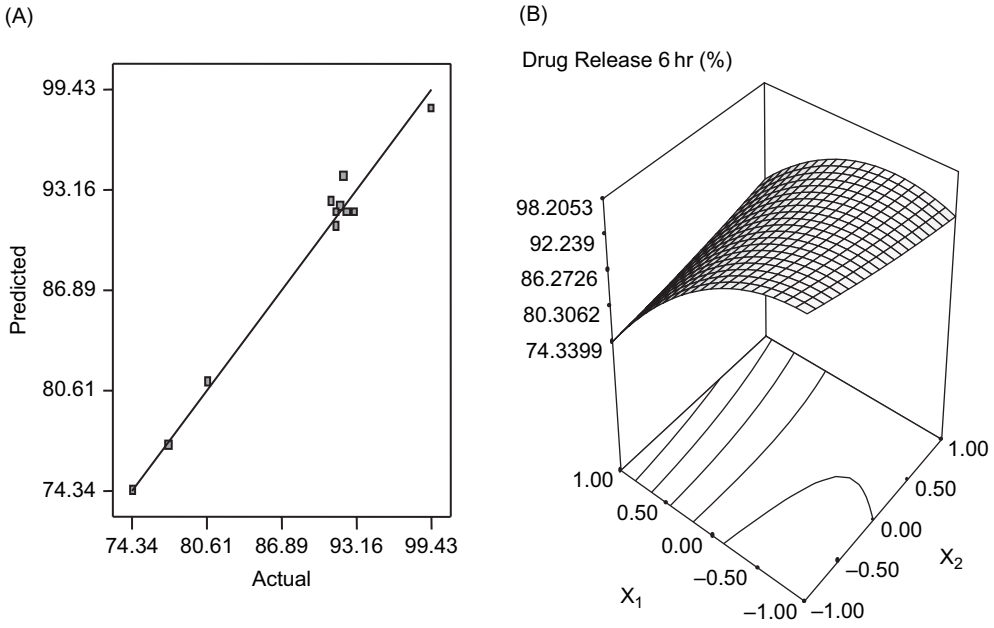

FIGURE 7. (A) Correlation between actual and predicted values. (B) Response surface plot showing the effect of X_1 and X_2 on the response drug release 6 hr (Y_3).

also increased. At higher pH values, alginate forms a soluble salt, thus providing less resistance to erosion and the release mechanism predominantly changes from diffusion controlled to erosion controlled (Streubel, Siepmann, & Bodmeier, 2000). The interaction between factors X_1 and X_2 can be elucidated by using response surface plot as illustrated in Figure 8. If X_1 was increased from –1 to +1 level by keeping X_2 at lower level, the effect on diffusion exponent was found to be minimal. And if X_2 was at higher level, the same diffusion exponent value increased from 0.88 to 1.25 by increasing the factor X_1 from –1 to +1 level. The probable explanation for this behavior may be due to increased polymer load in the delivery system, where the system

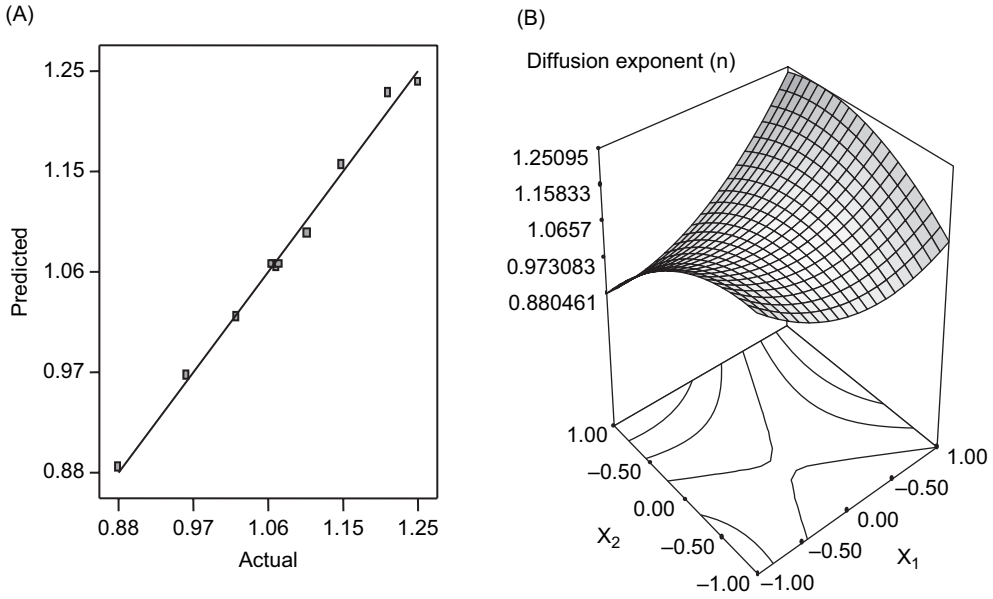

FIGURE 8. (A) Correlation between actual and predicted values. (B) Response surface plot showing the effect of X_1 and X_2 on the response diffusion exponent (Y_3).

takes a complete control on the release of gliclazide due to polymer chain relaxation and disentanglement leading to erosion (Li, Lin, Daggy, Mirchandani, & Chein, 2001). High level of X_2 shows high value of $T_{50\%}$ (Y_5) at all the levels of X_1, thus indicating that as the amount of polymer increased in the tablet, it causes quicker hydration of polymers (at different pH) to form a viscous layer around the tablet and thus delay the drug release from the dosage form with simultaneous increase in $T_{50\%}$ values.

Optimization

The data of pure error and lack of fit are summarized in Table 7. Multiple response optimization approach was considered more useful and suitable for optimizing hardness and release properties from extended release tablet dosage form. To optimize 5 responses with different targets, a multi-criteria decision approach like numerical optimization technique by the desirability function was used to generate the optimum settings for the formulation (Sanchez-Lafuente et al., 2002). The variables were optimized for the response Y_1–Y_5 and the optimized experimental parameters were arrived at by maximizing the hardness (Y_1) with constraints on release properties ($11 \le Y_2 \le 13.3$, $75 \le Y_3 \le 85$, $0.9 \le Y_4 \le 1.0$, $3.5 \le Y_5 \le 4.0$) to exclude out layers, if any. The desirability function response plot is shown in Figure 9. The optimized formulation (OPF) comprises 8.00 mg of keltone®-HVCR and 14.10 mg of eudragit®-EPO with a desirability of 0.9176. Even though, to challenge the reliability of the response surface model, new optimized combination was prepared according to the predicted model and evaluated for the responses. The results in the Table 8 showed a good relationship between the experimented and predicted values, which confirmed the practicability and validity of the model.

SEM Studies

The surface and cross-sectional SEM images of optimized matrix formulation are shown in Figure 10. Figure 10a shows the intactness of the tablet before hydration and the smaller pores though which the drug diffuses, while Figure 10b shows the cross-sectional images indicated by network of highly porous structure, which would probably explain the routes for the drug to diffuse with in the body of the gel layer and the surface erosion of the gel layer.

Stability Studies

Stability studies were performed under accelerated storage conditions as per ICH guidelines. The drug content and hardness of optimized formulation before and after 6 months stability studies were subjected to statistical analysis using the paired *t*-test and the results show that they are statistically significant ($P > 0.05$) as indicated in Table 9. The in vitro drug release profiles of optimized formulation before and after stability studies are illustrated in Figure 11. The profiles appeared to be almost super-imposable. The calculated $f2$ values obtained in this study for optimized formulation OPF before stability studies versus after stability studies at 30°C/65RH and 40°C/75RH were 91.85 and 75.36, respectively. These findings suggest that the in vitro drug release profiles investigated were therefore similar.

TABLE 7
Summary of ANOVA Results in Analyzing Lack of Fit (LOF) and Pure Error

Source	*SS*	*df*	*MS*	*F* Value	*p* > *F*
Hardness (kg/cm^2)					
Model	1.19	5	0.24	196.170	< 0.0001
Residual	0.006044	5	0.00120	–	–
Total	1.19	10	–	–	–
Lack of fit	0.00544	3	0.00181	6.05	0.1452
Pure error	0.00060	2	0.00030	–	–
Drug release at 1 hr (%)					
Model	19.29831	5	3.859661	20.70302	0.0024
Residual	0.932149	5	0.18643	–	–
Total	20.23045	10	–	–	–
Lack of fit	0.717149	3	0.23905	2.223718	0.3252
Pure error	0.215	2	0.1075	–	–
Drug release at 6 hr (%)					
Model	572.5598	5	114.512	58.35117	0.0002
Residual	9.812311	5	1.962462	–	–
Total	582.3721	10	–	–	–
Lack of fit	8.663627	3	2.887876	5.028147	0.1704
Pure error	1.148684	2	0.574342	–	–
Releae exponent (n)					
Model	0.110029	5	0.022006	137.2045	< 0.0001
Residual	0.000802	5	0.00016	–	–
Total	0.110831	10	–	–	–
Lack of fit	0.000753	3	0.000251	10.31873	0.0896
Pure error	4.87E-05	2	2.43E-05	–	–
$T_{50\%}$ (hr)					
Model	0.590117	5	0.118023	231.2246	< 0.0001
Residual	0.002552	5	0.00051	–	–
Total	0.592669	10	–	–	–
Lack of fit	0.002304	3	0.000768	6.177007	0.1425
Pure error	0.000249	2	0.000124	–	–

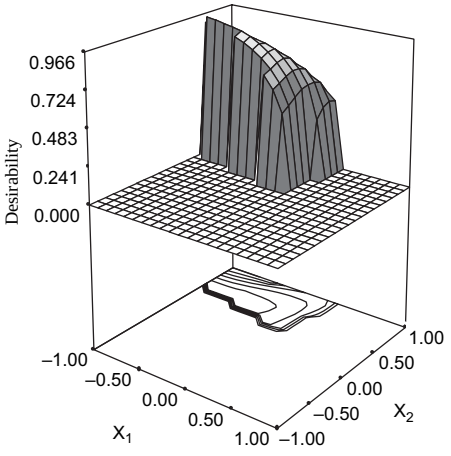

FIGURE 9. Desirability response surface plot.

TABLE 8
Comparison Between Experimented (E) and Predicted (P) for the Most Predicted Optimal Formulation OPF

	Optimized Formulation (OPF)	
Dependent Variables	E*	*P*
Hardness (kg/cm^2)	4.77 ± 0.058	4.72
Drug release at 1 hr (%)	12.65 ± 0.805	12.3
Drug release at 6 hr (%)	83.94 ± 0.605	85.0
Diffusion exponent (*n*)	0.91 ± 0.04	1.0
$T_{50\%}$ (hr)	3.55 ± 0.06	3.50

*All the values are expressed as $M \pm SD$ of three readings.

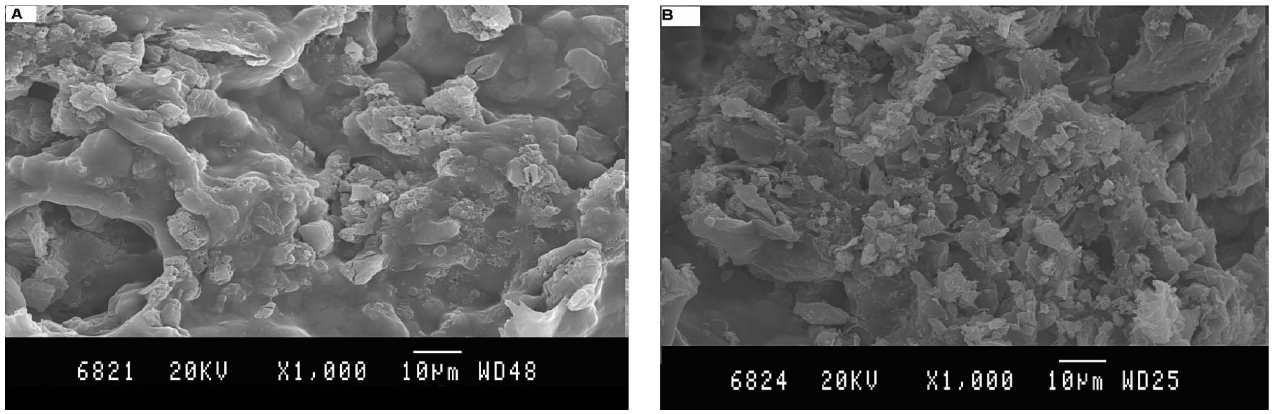

FIGURE 10. (A) The surface and (B) The cross-sectional SEM images of optimized matrix formulation.

TABLE 9
Change in Drug content and Hardness of OPF
After 180 Days Stability Studies

Type	Drug Content* (%)	Hardness* (kg/cm^2)
OPF at 30°C/65%RH	100.77 ± 0.136	4.60 ± 0.173
OPF at 40°C/75%RH	99.60 ± 0.172	4.33 ± 0.208
Paired *t*-test	*P* value	
OPF vs 30°C/65%RH	0.065	0.208
OPF vs 40°C/75%RH	0.071	0.147
30°C/65%RH vs 40°C/75%RH	0.099	0.121

*All the values are expressed as $M \pm SD$ of three readings.

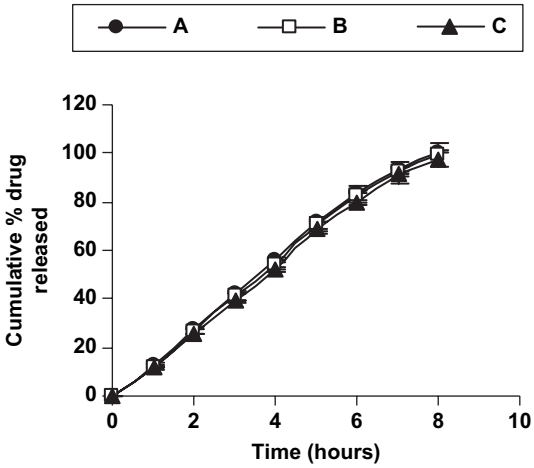

FIGURE 11. Comparison of release profiles of optimized formulation (OPF) before and after stability studies. A = release profiles of OPF at the beginning of the stability studies; B and C = release profiles obtained at the end of 6 months stability studies at various temperature and humidity conditions. B = 30°C ± 2°C/65% ± 5% RH; C = 40°C ± 2°C/75% ± 5% RH. All the values are expressed as $M \pm SD$ of three readings.

CONCLUSION

A central composite design (face centered alpha 1) was performed to study the effect of formulation variables on hardness and release properties by applying computer optimization technique. Amount of eudragit®-EPO along with its interaction with amount of keltone®-HVCR was found to be significantly affecting the studied response variables indicating that an appropriate balance between the various levels of studied independent variables is important to acquire proper control to yield optimal hardness and release properties. The mechanism of drug release from the optimized formulation was confirmed as zero-order release pattern. The statistical approach for formulation optimization is a useful tool, particularly in simultaneously evaluating several variables. Observed responses were in close agreement with the predicted values of the optimized formulation, there by demonstrating the feasibility of the optimization procedure in developing gliclazide extended release tablets.

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