Design, development and optimization of multiple-unit buoyant beads containing Pioglitazone.

[1]Jadupati Malakar, [2]Mithun Bhowmick, [3]Pratibha Bhowmick

Bengal college of pharmaceutical sciences and research. Durgapur-713212, West Bengal, India
Author for correspondence: Jadupati Malakar

Abstract:
In the current work, formulation optimization of multiple-unit Pioglitazone-loaded buoyant beads was using various polymers like low methoxyl pectin (methoxyl content less than 7%), Poloxamer 407 and magnesium stearate as low density materials as independent variables and their effects on drug entrapment efficiency (DEE, %) and cumulative drug release after 8 h (R_{8h}, %) as responses were analyzed after performing a full 2^3 factorial design. The optimized formula for the preparation of buoyant beads containing Pioglitazone was found 749.98 mg low methoxyl pectin, 345.43 mg Poloxamer 407 and 166.33 mg magnesium stearate. The average diameter, density values, and DEE (%) of these dried beads containing Pioglitazone ranged from 1.67 ± 0.05 to 1.86 ± 0.07 mm, 0.901 ± 0.08 to 0.984 ± 0.09 gm/cm^3 and 66.21 ± 2.72 to 75.43 ± 2.15%. These optimized formulations of beads were characterized by scanning electron microscopy and FTIR spectroscopy. These formulated beads containing Pioglitazone were showed buoyancy as well as sustained release over 8 h, *in vitro*.

Keywords: Pioglitazone; Buoyant beads; Multiple-unit; Sustained release; 2^3 factorial designs.

Introduction:

Pioglitazone, oral antidiabetic drug, chemically is (±)-5-{p- [2-5(-ethyl-2-pyridyl) ethoxy] benzyl}-2, 4-thiazolidinedione.It is used in the management of type II diabetic mellitus [1]. It is rapidly absorbed after oral doses and it has a short biological half life (approx. 3-5 h), which makes it suitable candidate for sustain or controlled delivery system [1, 2]. In last few decades', many sustained or controlled delivery system have been investigated and developed for oral administration, which are amenable to deliver the drugs(s) into systemic circulation offering many advantages *viz*., ease of administration, patient compliance and economical, *etc*., and also ensure better clinical effect along with minimum dosing frequency [3]. As conventional drug delivery systems are not sufficient enough to increase gastric residence time in the stomach and proximal portion of small intestine that results incomplete drug release at the absorption site in the gastrointestinal tract (GIT) lowering the efficacy of administered dose [4].Therefore, oral sustained or controlled drug delivery systems necessitate be designing and formulating to remain the system in stomach for several hours to augment the bioavailability of candidate drug [3,5,6]. In gastro-retentive drug delivery systems, various approaches have been reported in literature for better improvement of gastro-retention in oral dosage forms, *viz*., floatation [7-12], mucoadhesion [13-22], combination of floatation-mucoadhesion [23,24], sedimentation [25], unfoldable, expandable, or swellable systems[26], super porous hydrogel systems[27] and magnetic systems [28], *etc*.

Now a days, multiple-unit gastro-retentive drug delivery systems have extensively accepted over single-unit system in pharmaceutical research because they seem to be an alternative approach and they have shown to trim down inter-intra subject variability in drug absorption with lowering the possibilities of dose-dumping characteristic, whereas single-unit gastro-retentive systems such as tablets or capsules may exhibit the all or none emptying phenomenon [29, 30]. Multiple-unit floating systems are cautiously formulated to get remained buoyant on gastric fluid as they acquire low bulk density compared to aqueous medium and yields slow drug release for prolonged period of time in stomach [11, 30]. The buoyancy of developed multiple-unit floating beads solely depends on the incorporated low density materials [11]. Some multiple-unit floating beads have been developed in recent time consisting of low density oils like mineral oil, olive oil, linseed oil, sunflower oil, groundnut oil, castor oil and menthe oil, *etc* [10, 12, 30, 31]. However, the incorporation of low density material like magnesium stearate only instead of above mentioned oils may be an interesting thought or an alternative to get buoyant alginate gel beads [11]. The optimization in pharmaceutical formulations needs to have appropriate combination of independent process variables that will influence the responses of ultimate product that it is a huge task to the formulators and researchers. None the less, this can be easily analyzed and understood using established statistical design of experiment tools such as factorial designs are considered the most effective in estimating the influence of individual process variables with minimum experimentation and time where all factors are studied in all possible combinations [10]. In optimization, experimental design allows a sensitive relationship on variables and responses and how formulation variables can influence the product quality in a defined design space which signifies interaction of input variables and process parameter through a mathematical means [32]. Response surface methodology is extensively employed to optimize the formulations by securing a better understanding of the process or design in establishing the robustness of the formulation [33, 34]. In this investigation, we aimed to formulate and optimize the best formulation of multiple-unit Pioglitazone loaded beads using various polymers like low methoxyl pectin (methoxyl content less than 7%), Poloxamer 407 and magnesium stearate as low density materials as independent variables and their effects on drug entrapment efficiency (DEE, %) and cumulative drug release after 8 h (R_{8h}, %) as responses after performing a full 2^3 factorial model.

Materials and methods:

Materials

Pioglitazone was purchased from B. S. Traders Pvt. Ltd., India, low methoxyl pectin(B. S. Traders Pvt. Ltd., India).Poloxamer 407 (a gift sample from Aurobindo Pharma Limited Research Centre II, Telengana), magnesium stearate (Loba Chemie., India) and zinc chloride (Merck Ltd., India) were used. All other chemicals and reagents used were of analytical grade.

Methods

Preparation of beads containing Pioglitazone

The buoyant beads containing Pioglitazone were prepared by ionotropic-gelation method. Briefly, required amount of pectin was dissolved in 20 ml demineralised water with constant stirring. Required amount of Poloxamer 407 and magnesium stearate, 100 mg of Pioglitazone were added to pectin dispersion. The final mixture containing pectin, Poloxamer 407 and magnesium stearate was well-stirred at 5000 rpm continuously for 30 min until the homogeneous and stable suspension was formed. Then, the suspension was dropped through 23G needle into 10 % (w/v) zinc chloride solution (100 ml), and the added droplets were retained for 15 min in the zinc chloride solution to complete the curing reaction. The prepared beads were filtered. The dried beads containing Pioglitazone were stored in desiccators until used.

Experimental design

A 2^3 full factorial design was employed for experimental design where three factor underlying the different weight masses of pectin (X_1), Poloxamer 407 (X_2) and magnesium stearate (X_3) as three selected independent variables which were further varied at two, *i.e.*, low level (-1) and high level (+1). The coded values were applied after performing the preliminary trials and are shown in **Table 1.** The drug entrapment efficiency (DEE, %) and cumulative drug release (R_{8h}, %) were measured as dependant variable and their dependency on independent variables and statistical experimentation was extensively performed using Design Expert 8.0.6.1 software. Responses are shown in **Table 2.**

Determination of DEE (%)

Accurately weighed 100 mg of prepared beads containing Pioglitazone from each batch were taken separately and were crushed using clean pestle-mortar. The crushed powders were placed in 100ml of 0.1N HCl (pH 1.2) and kept for 24h with occasionally shaking at 37±0.5°C. After the stipulated time, the mixture was stirred at 500 rpm for 15min on a magnetic stirrer. The polymer debris formed after disintegration of bead was removed by filtering through Whatman® filter paper (No. 40). Then, the drug content in the filtrate samples were determined using a UV–Visible spectrophotometer (Thermo Spectronic UV-1, USA) by measuring absorbance at λ_{max} of 269.5 nm. DEE (%) of beads was calculated using this following formula [1]:

DEE (%) = (actual drug content in beads/ theoretical drug content in beads) × 100

Determination of bead size

Diameters of dried beads containing Pioglitazone were measured using digital slide calipers (Mitutoyo Corporation, Japan) by inserting the beads in between the space of two metallic plates and diameter of resultant beads were displayed in the digital screen of the previously calibrated equipment. The average size was then calculated by measuring the diameter of 3 sets of 20 beads from each batch.

Determination of density

The mean weights and diameters of these beads containing Pioglitazone were measured and used to calculate densities of beads using the following equations [10]:

$\rho = M/V$, and $V=4/3.\ \pi r^3$

Where ρ, *M*, *V*, and *r* are the density (g/cm^3), weight (g), volume (cm^3)and radius (cm) of the beads, respectively

Surface morphology analysis

For the morphological study, beads containing Pioglitazone were mounted on a brass stub using double–sided adhesive tape coated with gold under a vacuum. Surface morphologies of beads containing Pioglitazone were examined by scanning electron microscope (SEM) (JSM-5310LV Scanning Microscope, Japan) at 15 kV.

Fourier transform-infrared spectroscopy (FTIR) analysis

Samples were tested as potassium bromide pellets by a FTIR spectroscope instrument (Alpha-FTIR, Bruker Optics, Germany). The pellets containing samples were placed in the sample holder of FTIR spectroscope instrument, individually. The spectral scanning was recorded over the range of 4000–600 cm^{-1}.

***In vitro* buoyancy evaluation**

The buoyant ability of formulated beads containing Pioglitazone was determined using dissolution apparatus type-II (Campbell Electronics, India). 50 beads were placed in the dissolution vessel containing 500 ml of simulated gastric fluid (pH 1.2) maintained at 37 ± 0.5°C for 7 h and the paddles were rotated at 50 rpm. The floating ability of beads was measured by visual observation. The time taken to buoyant at the surface of dissolution medium (known as buoyant lag-time) and duration of floating were noted [10, 11].

***In vitro* drug release studies**

The prepared beads containing Pioglitazone were evaluated for their drug release study in dissolution apparatus type-II (Campbell Electronics, India). An equivalent weight of beads containing 100 mg Pioglitazone was placed into 900 ml of simulated gastric fluid (pH 1.2), maintained at 37±0.5°C and 50 rpm paddle speed. 5ml of aliquots was collected at regular selected time intervals and soon after same amount fresh buffer was into dissolution vessel keeping the sink condition throughout the experiment. The collected aliquots were analyzed to find out the amount of drug release from the beads using a UV–Visible spectrophotometer (Thermo Spectronic UV-1, USA) by measuring absorbance at λ_{max} of 269.5 nm.

Analysis of *in vitro* drug release kinetics and mechanism

To analyze the mechanism of drug release from these beads containing Pioglitazone, the *in vitro* dissolution data were fitted to various mathematical models like zero order, first order, Higuchi and Korsmeyer-Peppas models [35-37].

Zero-order Model: $F = K_0 t$, where F represents the fraction of drug released in time t and K_0 is the apparent release rate constant or zero-order release constant.

First-order Model: ln (1-F) = - K_{1st} t, 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 t, 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 release exponent, this indicates the drug release mechanism.

Again the Korsmeyer-Peppas model has been employed in the *in vitro* drug release behavior analysis of various pharmaceutical formulations to make a distinction between various release mechanisms: Fickian release (diffusion-controlled release), non-Fickian release (anomalous transport) and case-II transport (relaxation- controlled release). When, $n \leq 0.5$, the release is Fickian. The n value between 0.5 and 1.0 is defined as non-Fickian release. When, $n \geq 1.0$, it is case-II transport and this involves polymer dissolution and polymeric chain enlargement or relaxation [38-40].

Statistical analysis

Statistical optimization was performed using Design-Expert 8.0.6.1 software (Stat-Ease Inc., USA). All measured data are expressed as mean± standard deviation (S.D.) with simple statistics.

Results and discussion:

Optimization

A full 2^3 factorial design was performed along with three independent variables, *viz*., pectin (X_1), Poloxamer 407 (X_2) and magnesium stearate (X_3) which were varied at two levels as high and low.

Table 1: Composition of various coded values used in 2^3 factorial designs

Coded values		Independent variables (actual values)		
		Pectin [X_1] (mg)	**Polaxamer 407 [X_2] (mg)**	**Mg-stearate [X_3] (mg)**
Levels	**Low (-1)**	400	100	50
	High (+1)	600	200	100

Their influences on the responses like DEE (%) and R_{8h} (%) in simulated gastric fluid (pH 1.2) were comprehensively investigated in the current study using Design Expert 8.0.6.1 software, which yield a first order polynomial equation consisting of a suitable correlation between the main factors (variables) and their interaction on obtained responses. The equation is given below:

$$Y = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_3 + b_4 X_1 X_2 + b_5 X_1 X_3 + b_6 X_2 X_3 + b_7 X_1 X_2 X_3$$

Where Y = dependent variable, while b_0 = intercept, b_1, b_2, b_3, b_4, b_5, b_6 and b_7 = regression coefficient; X_1, X_2 and X_3 = main factors; X_1X_2, X_1X_3 and X_2X_3 = interactions between main factors.

In this study, a total 8 trial of beads containing Pioglitazone were designed and formulated and their relevant responses are depicted **Table 2**.

Table 2: 2^3 factorial designs and their observed response value along with coded values in brackets

F code	Pectin [X_1] (mg)	Polaxamer 407 [X_2] (mg)	Mg-stearate [X_3] (mg)	Responses	
				DEE (%)[a,c]	**R_{8h} (%)[b,c]**
F-1	600.00 (+1)	200.00 (+1)	100.00 (+1)	75.43 ± 2.15	46.45 ± 2.26
F-2	600.00 (+1)	100.00 (-1)	50.00 (-1)	70.82 ± 3.05	50.02 ± 2.12
F-3	600.00 (+1)	100.00 (-1)	100.00 (+1)	72.44 ± 2.09	49.41 ± 3.14
F-4	600.00 (+1)	200.00 (+1)	50.00 (-1)	72.56 ± 2.55	51.42 ±2.75
F-5	400.00 (-1)	100.00 (-1)	50.00 (-1)	66.21 ± 2.72	57.73 ± 2.21
F-6	400.00 (-1)	200.00 (+1)	100.00 (+1)	70.30 ± 2.42	44.54 ± 1.95
F-7	400.00 (-1)	100.00 (-1)	100.00 (+1)	67.62 ± 3.05	55.57 ± 2.32
F-8	400.00 (-1)	200.00 (+1)	50.00 (-1)	67.82 ± 2.32	50.52 ± 1.74

(+1) = higher value, (-1) = lower value.

[a]DEE = drug entrapment efficiency.

[b] R_{8h}(%) = cumulative drug release after 8h.

[c]Mean ± S.D.: n=3.

The model polynomial equation relevant to DEE (%) as response was obtained:

$$DEE\ (\%) = 56.25 + 2.04 \times 10^{-2} X_1 + 2.50 \times 10^{-5} X_2 - 7.15 \times 10^{-3} X_3 + 1.02 \times 10^{-5} X_1 X_2 + 2.85 \times 10^{-5} X_1 X_3 + 2.33 \times 10^{-4} X_2 X_3$$

Another polynomial equation relevant to R_{8h} (%) as response was obtained:

$$R_{8h}\ (\%) = 97.06 - 8.5 \times 10^{-2} X_1 - 9.6 \times 10^{-2} X_2 - 9.90 \times 10^{-3} X_3 + 4.17 \times 10^{-4} X_1 X_2 \; 1.28 \times 10^{-4} X_1 X_3 - 8.18 \times 10^{-4} X_2 X_3$$

Data obtained from the experimentation were subjected into the "ANOVA" study which assured that all models were found to be significant ($p<0.05$) for all responses shown in **Table 3.**

Table 3: Summary of ANOVA for response parameters

Source	Sum of square	d.f.[a]	Mean square	*F*-value	*p*-Value prob >F

For DEE (%)[b]					
Model	66.33	6	11.06	3060.30	0.0138 (S)
X_1	46.51	1	46.51	12875.57	0.0056 (S)
X_2	10.24	1	10.24	2834.00	0.0120 (S)
X_3	8.84	1	8.84	2447.34	0.0129 (S)
X_1X_2	2.1×10^{-2}	1	2.1×10^{-2}	5.82	0.2502 (NS)
X_1X_3	4.1×10^{-2}	1	4.1×10^{-2}	11.24	0.1845 (NS)
X_2X_3	0.68	1	0.68	187.85	0.0464 (S)
For R_{8h}(%)[c]					
Model	131.79	6	21.96	602.59	0.0312 (S)
X_1	15.29	1	15.29	419.49	0.0311 (S)
X_2	49.00	1	49.00	1344.44	0.0174 (S)
X_3	23.53	1	23.53	645.54	0.0250 (S)
X_1X_2	34.78	1	34.78	954.12	0.0206 (S)
X_1X_3	0.82	1	0.82	22..47	0.1323 (NS)
X_2X_3	8.36	1	8.36	229.47	0.0420 (S)

X_1, X_2 and X_3 represent the amount of pectin, Poloxamer 407 and magnesium stearate in mg, respectively; X_1X_2, X_1X_3 and X_2X_3 are their interaction effects.

S and NS indicate significant and non significant, respectively.

[a]d.f. indicatesdegree of freedom.

[b]DEE (%) indicates drug entrapment efficiency.

[c]R_{8h} (%) indicates cumulative drug release after 8h.

The final equation was confirmed by eliminating the non-significant term ($p>0.05$) in polynomial equation [24] and they are:

$$\text{DEE (\%)} = 56.25 + 2.04\times10^{-2}X_1 + 2.50\times10^{-5}X_2 - 7.15\times10^{-3}X_3 + 2.33\times10^{-4}X_2X_3$$

$$R_{8h}\ (\%) = 97.06 - 8.5\times10^{-2}X_1 - 9.6\times10^{-2}X_2 - 9.90\times10^{-3}X_3 + 4.17\times10^{-4}X_1X_2 - 8.18\times10^{-4}X_2X_3$$

The Pareto chart reveals the significance of each response coefficient as shown in **Fig. 1** and **Fig. 2**. In Pareto chart, coefficient is significant if t-values effects above the Bonferroni line, whereas coefficients with t values of effects in between Bonferroni line and t limit line are termed as coefficients likely to be Significant, but coefficient gets insignificant for t-values that effects below the t limit line. However, these charts became functional to establish ANOVA results for easy comprehensible one throughout elimination insignificant ($p<0.05$) terms from both polynomial equations [36].

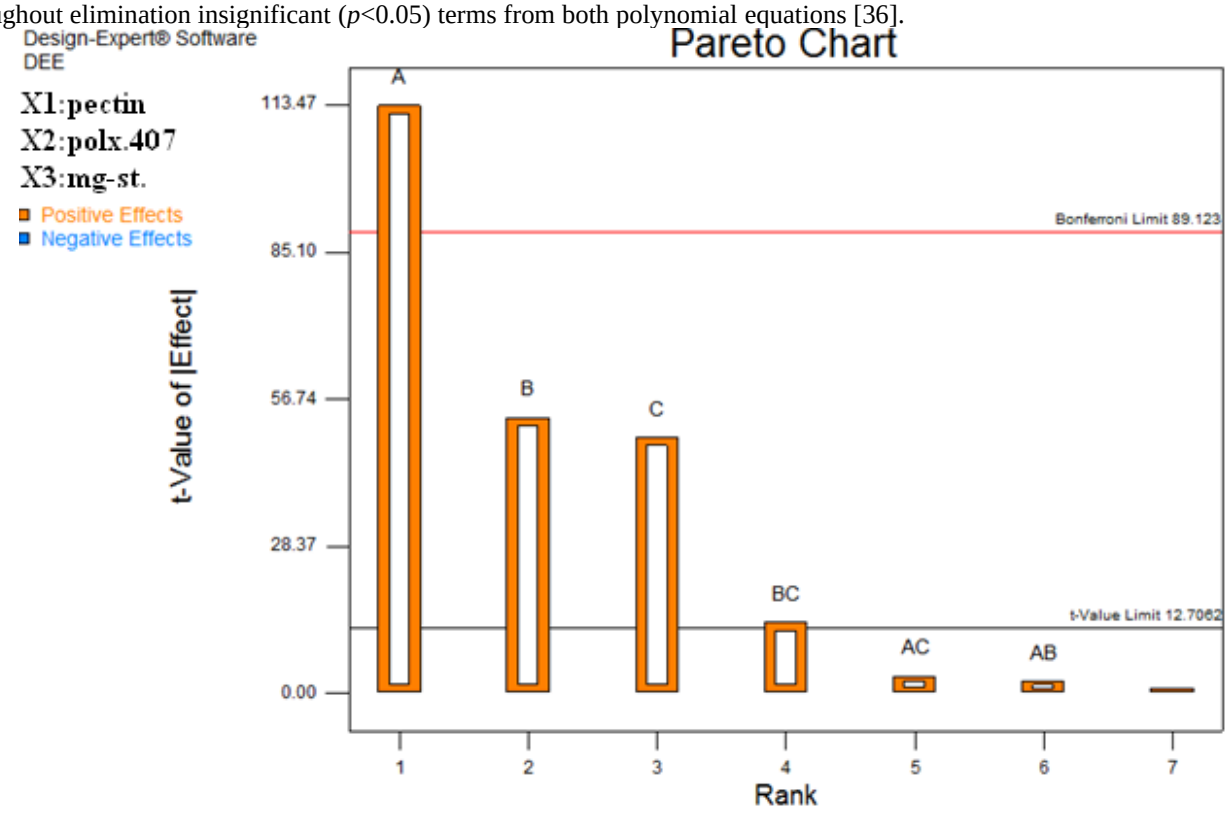

Fig. 1: Pareto chart for DEE (%).

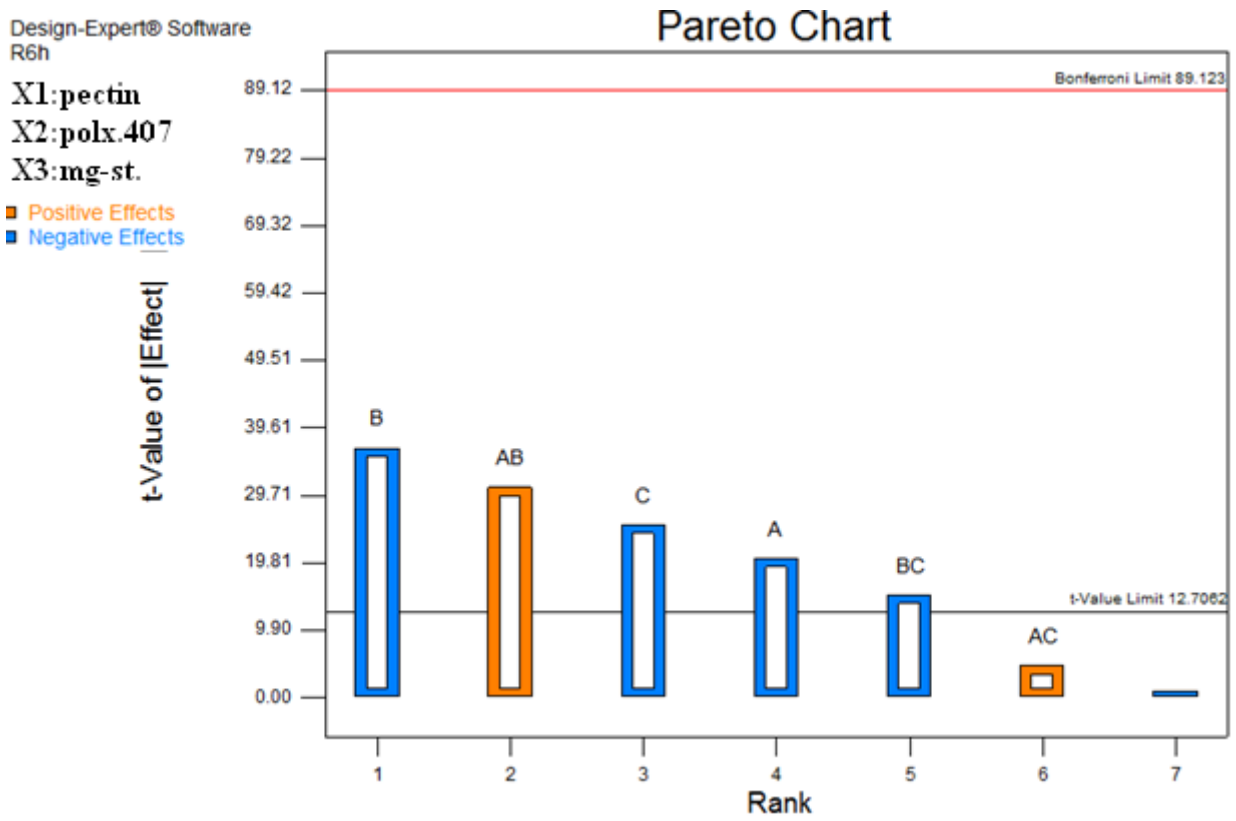

Fig. 2: Pareto chart for R_{8h} (%).

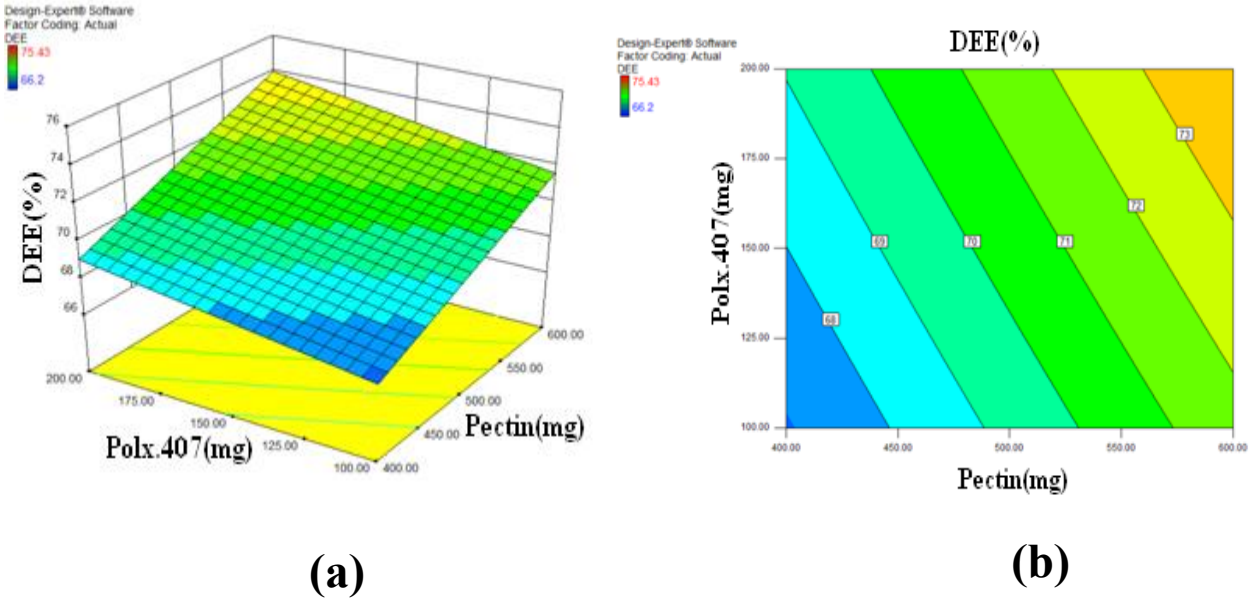

Fig. 3: Effects of amounts of pectin and Poloxamer 407 on DEE (%) shown by response surface plot (a) and contour plot (b).

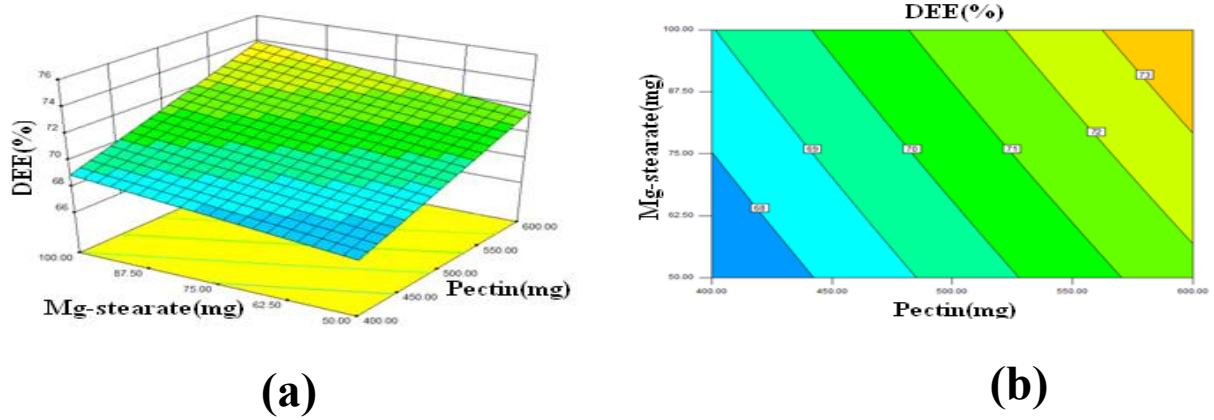

Fig. 4: Effects of amounts of pectin and magnesium stearate on DEE (%) shown by response surface plot (a) and contour plot (b).

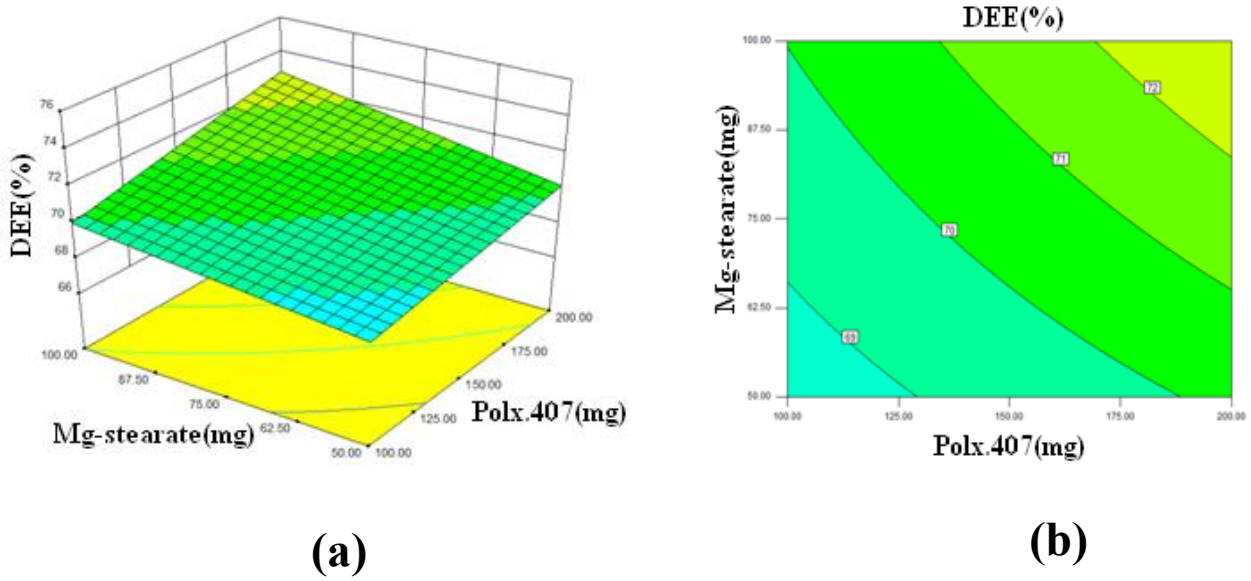

Fig. 5: Effects of amounts of Poloxamer 407 and magnesium stearate on DEE (%) shown by response surface plot (a) and contour plot (b).

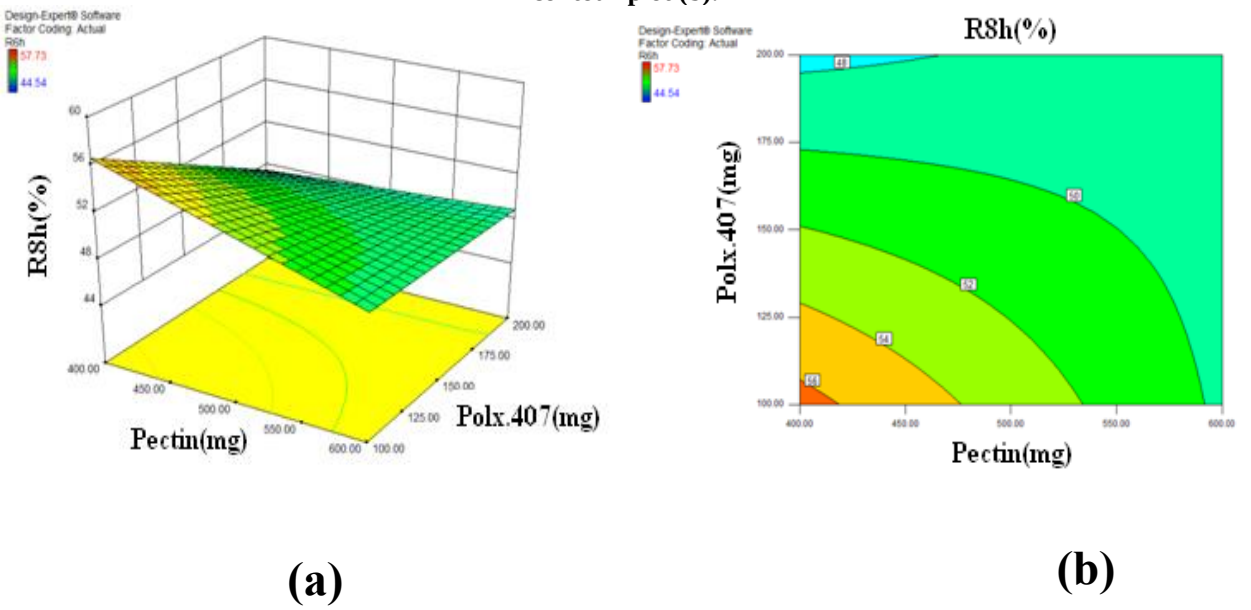

Fig. 6: Effects of amounts of pectin and Poloxamer 407 on R_{8h} (%) shown by response surface plot (a) and contour plot (b).

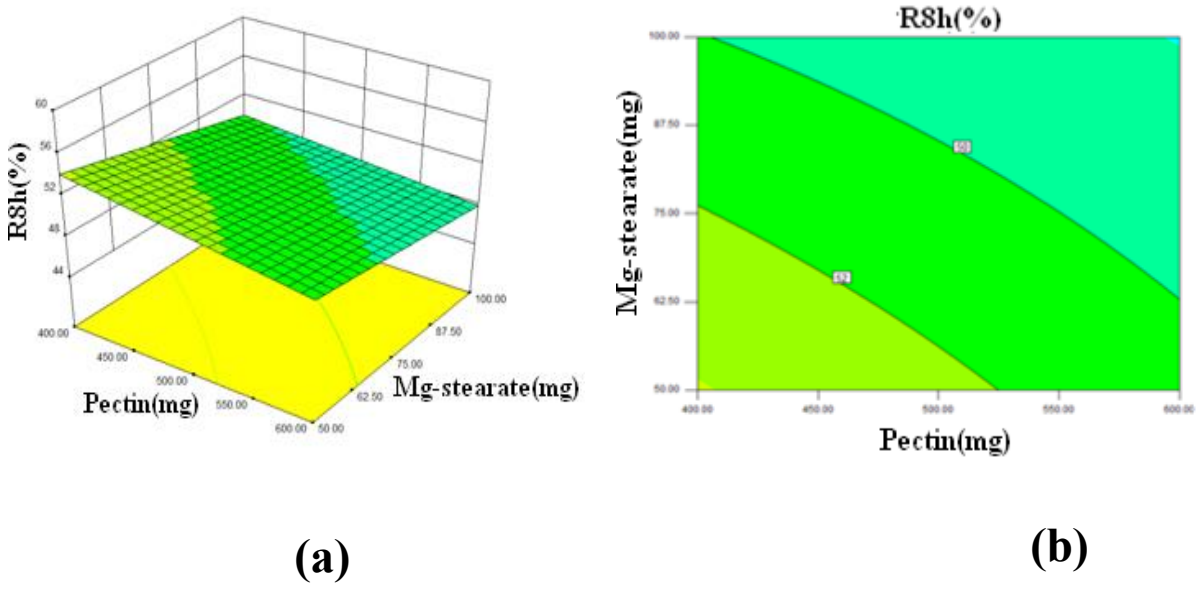

Fig. 7: Effects of amounts of pectin and magnesium stearate on R_{8h} (%) shown by response surface plot (a) and contour plot (b).

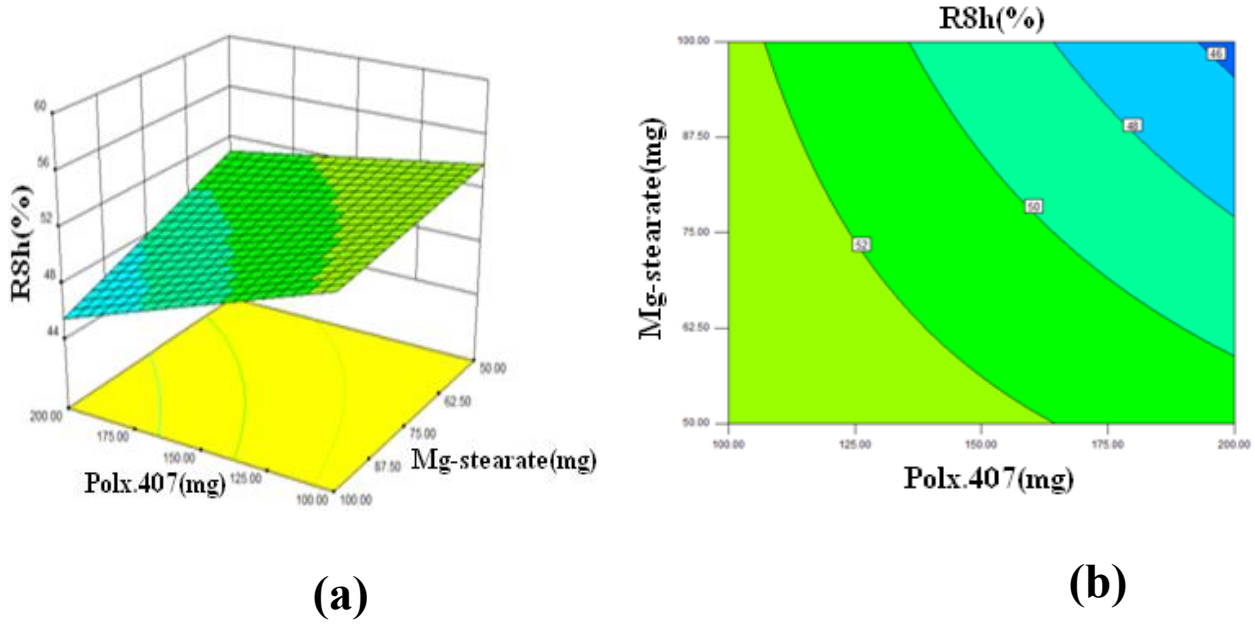

Fig. 8: Effects of amounts of Poloxamer 407 and magnesium stearate on R_{8h} (%) shown by response surface plot (a) and contour plot (b).

The effect of three individual factors (X_1, X_2 and X_3) and their interaction (X_1X_2, X_1X_3 and X_2X_3) on the DEE (%) were found positive in all respect but both effect were observed on R_{8h}(%) that are shown in **Fig.1–2**.The Design Expert 8.0.6.1 software provided three dimensional response surface plots and contour plots were shown in **Fig 3, 4 and 5** for DEE (%) and **Fig.6,7 and 8** for R_{8h}(%) release, respectively.

A numerical optimization technique based on the desirability approaches was employed to achieve new optimized formulation with desired responses. This adopted optimization technique also affirmed the desirable ranges of response parameters in which DEE were limited to 90≤ DEE≤ 100%,whereas drug release were limited as 40 ≤ R*8h*≤ 55%. In concern of variables, the ranges of factors were found as 600 ≤ X_1≤ 750, 200 ≤ X_2 ≤ 400 and 150 ≤ X_3 ≤ 200, respectively. The optimized desirable ranges for beads containing Pioglitazone were found to be; X_1=749.98 mg, X_2 = 345.43mg and X_3 = 166.33mg. The numerical analysis was performed to acquire the optimal values of responses based on desirability criterion by the help of Design Expert 8.0.6.1 software, which led to develop optimized (F-O) beads containing Pioglitazone with respect to DEE (%) andR_{8h} (%). **Table 3** and**4** depict the results of predicted values obtained from the mathematical model and same practically observed.

Table 4: Results of experiments to assure optimization capability.

F. Code	Pectin (mg) X_1	Poloxamer 407 (mg) X_2	Mg-stearate (mg) X_3	Responses	
				DEE (%) [a]	R_{8h} (%)[b]
F-O	749.98	345.43	166.33	Actual values[c]	

				88.82 ± 3.11	41.42 ± 3.07
				Predicted values	
				90.00	40.00
% Error[d]				-1.31	3.55

[a]DEE (%)= drug entrapment efficiency
[b] R_{8h} (%) = cumulative drug release after 8 h
[c]Actual values = Mean ± SD; n = 3
[d] Percentage of error (%) = (actual value-predicted value) / predicted value × 100

Bead size

The average diameter of these dried beads containing Pioglitazone ranged from 1.67 ± 0.05 to 1.86 ± 0.07 mm (**Table 5**).Increasing bead size was found with increasing of pectin and Polaxomer 407 amounts. Again, increasing bead size was evidenced with increasing of total amount of polymers used in preparation of beads containing Pioglitazone. This could be attributed due to the increase in viscosity of the blend with th1e increasing proportion of total polymers that in turn increased the droplet size during addition of the mixture to the cross linking solution.

Table 5: Density, particle size results of beads containing pioglitazone.

F code	Density[a] (gm/cm^3)	Diameter[a] (mm)
F-1	0.931 ± 0.05	1.86 ± 0.07
F-2	0.981 ± 0.07	1.80 ± 0.05
F-3	0.929 ± 0.07	1.82 ± 0.10
F-4	0.984 ± 0.09	1.85 ± 0.11
F-5	0.953 ± 0.03	1.67 ± 0.05
F-6	0.903 ± 0.05	1.74 ± 0.06
F-7	0.901 ± 0.08	1.68 ± 0.10
F-8	0.958 ± 0.03	1.72 ± 0.14

[c]Mean ± S.D.; n = 3

Density

Density values of various formulated beads containing Pioglitazone ranged from 0.901 ± 0.08 to 0.984 ± 0.09 gm/cm^3 (**Table 5**). The decreasing density of these beads was observed with the decreasing amount of polymer contents in these beads. However, this was also found to be decreased with increasing amount of magnesium stearate in beads formulation. The incorporation of low density materials was responsible to reduce the density of the formulated beads containing Pioglitazone.

Surface morphology analysis

Scanning electron microphotograph of the bead surface of optimized beads containing Pioglitazone showed a rough surface with small pores or channels (**Fig. 9**). The surface also had some wrinkles with grooves. No large crystals of drug were found on the bead surface, signifying the presence of drug particles as finely dispersed state in the polymeric matrix.

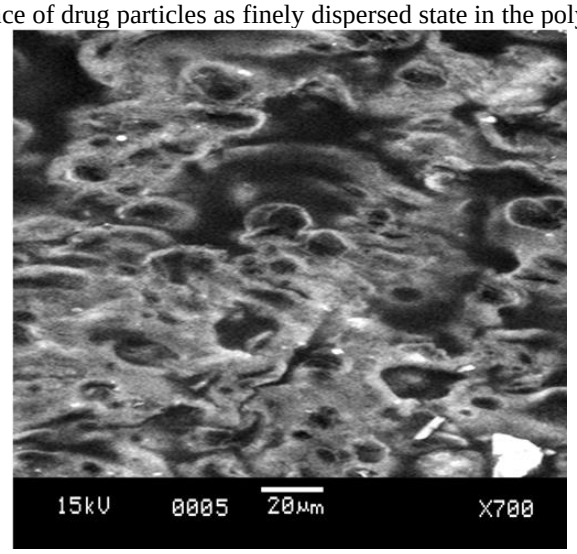

Fig. 9: Scanning electron microphotograph of the bead surface of optimized beads containing Pioglitazone showing rough surface with small pores or channels.

Drug entrapment efficiency

DEE (%) of formulated beads containing Pioglitazone were found in a range of 66.21±2.72 to 75.43±2.15%, which concludes that the drug entrapment in these beads was increased with increasing amount of pectin, Poloxamer 407 and magnesium stearate. The enhancement of DEE with increasing amount of pectin (X_1) in respective beads formulation occurred due to settlement higher amount of drug inside the polymeric $-Zn^{2+}$ cross linked gel network either physical interaction and entanglement or both between the drug and polymer. Poloxamer influenced the DEE by its inherent gelling properties that enable to hold the candidate

drug in its frame. The DEE was also increased with increasing amount of magnesium staerate that may be formation of lipophilic barrier, which restricted to passage of the drug from the formulation during preparation.

FTIR analysis

The FTIR spectra of pectin, Poloxamer 407, magnesium stearate, blank beads, beads containing Pioglitazone and Pioglitazone (drug) were presented **Fig. 10**.Various characteristics peaks and bands appeared in respective spectra of pectin; Poloxamer 407 and magnesium stearate were identified in the spectrum of blank beads (without Pioglitazone). In the FTIR spectrum of beads containing Pioglitazone, different characteristic peaks and bands appeared in respective spectra of blank beads and Pioglitazone(drug), were identified without any significant shifting so as tosuggesting the absence of any interaction in-between the drug (here Pioglitazone) and the excipients (namely pectin, Poloxamer 407, and magnesium stearate)in these low-density beads containing Pioglitazone.

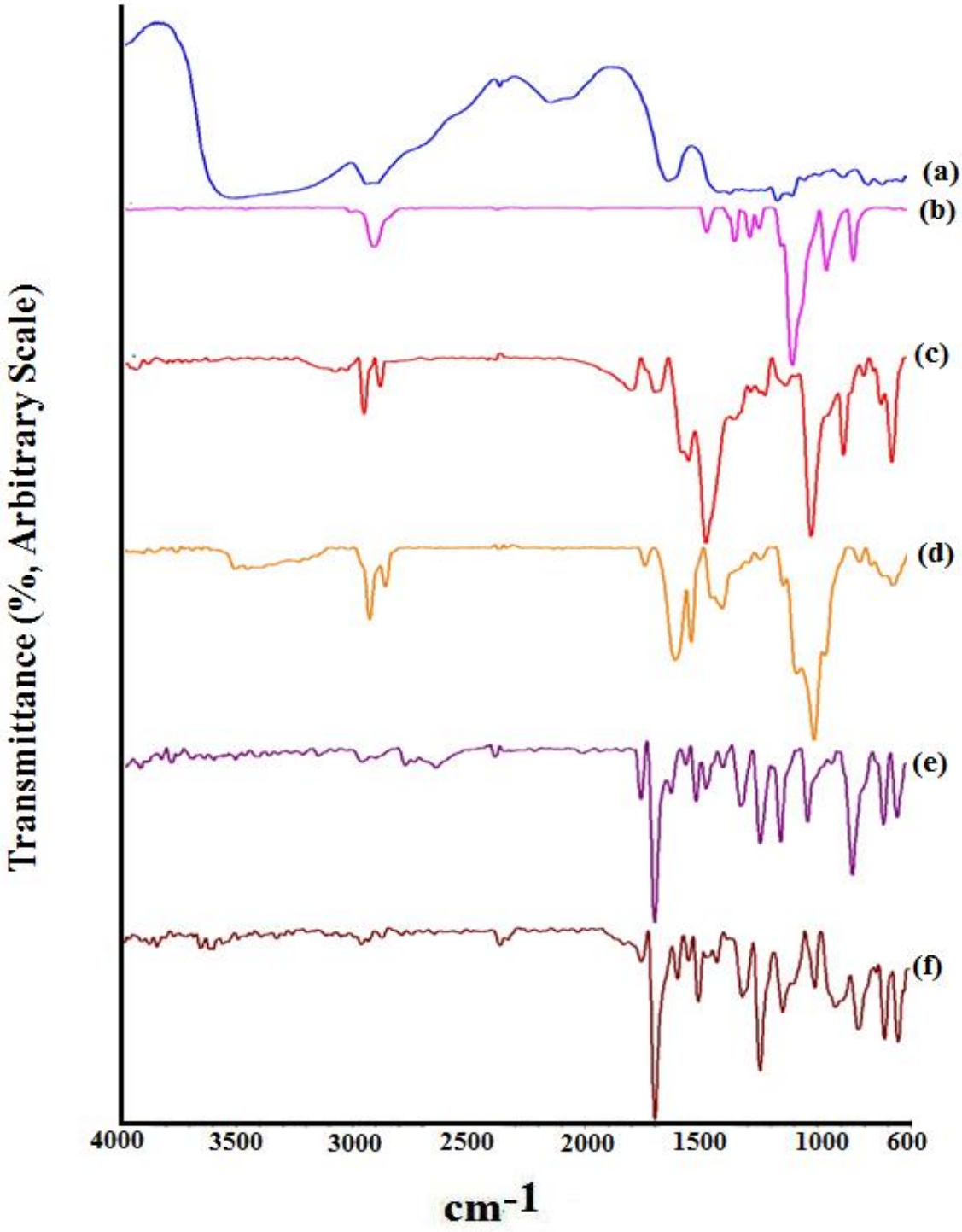

Fig. 10: FTIR spectra of pectin, Poloxamer 407, magnesium stearate, blank beads, beads containing Pioglitazone and Pioglitazone (drug).

***In vitro* buoyancy**

The *in vitro* floatation results of beads containing Pioglitazone in simulated gastric fluid (pH 1.2). The density values of all formulated beads were less than the density of in simulated gastric fluid (pH 1.2) (*i.e.*, 1.004 gm/cm^3) imparting their buoyancy (flotation) and these formulated beads containing Pioglitazone were showed buoyancy over 8 h. These buoyant beads were floated within 5 min after being placed in simulated gastric fluid at pH 1.2. The incorporation of Poloxamer 407 and magnesium stearate in these beads was responsible for floating. Thus, a clear correlation was evidenced between the density values of these beads containing Pioglitazone with their buoyancy and buoyant lag-time.

***In vitro* drug release**

In vitro drug release studies of all these buoyant beads containing Pioglitazone were performed in USP type II apparatus (Campbell Eletctronics, India) at 50 rpm maintained at 37± 0.5°C taking the dissolution medium of 900 ml of simulated gastric fluid (pH 1.2).The *in vitro* cumulative drug release from buoyant beads containing Pioglitazone (F-1 to F-8) in simulated gastric fluid (pH 1.2) is shown **Fig 11**. The cumulative drug release from these buoyant beads containing Pioglitazone was found in a range of 44.54±1.95 to 57.73±2.21%.The *in vitro* cumulative drug release from optimized buoyant beads containing Pioglitazone (F-O) in simulated gastric fluid (pH 1.2) is shown **Fig 12** with cumulative drug release of 41.72 ± 1.46% over 8 h. All formulation was shown sustained release over a time period of 8 h. Drug releases from the all formulation were found to be followed sustained release pattern over 8 h and it has been observed that drug release was being retarded in influence of increasing amount of three variables but most reasonable enlightenment of slow release could be attributed to integral of the pectin leading to shrink at the acidic medium(pH 1.2) and another one is related to magnesium stearate which generate lipophilic barrier to trim down the release by retarding the wetting properties of Pioglitazone buoyant bead formulations against the dissolution medium [41].

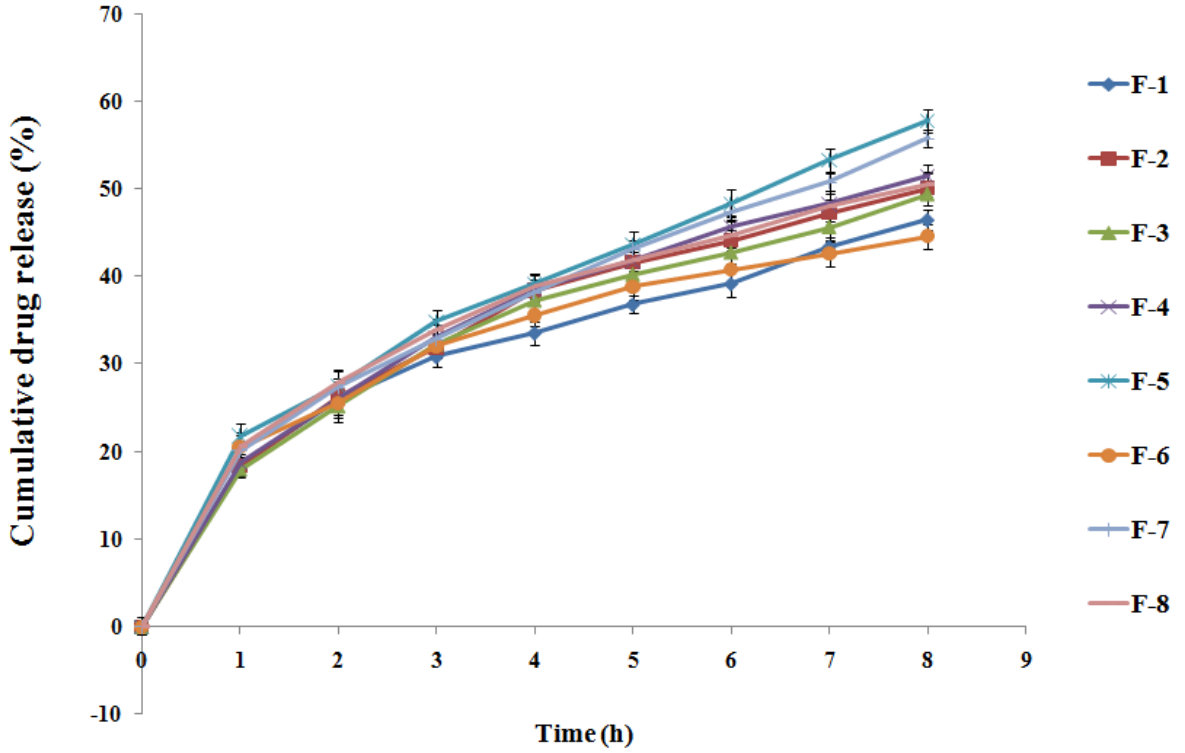

Fig. 11: The *in vitro* cumulative drug release from buoyant beads containing Pioglitazone (F-1 to F-8) in simulated gastric fluid (pH 1.2)

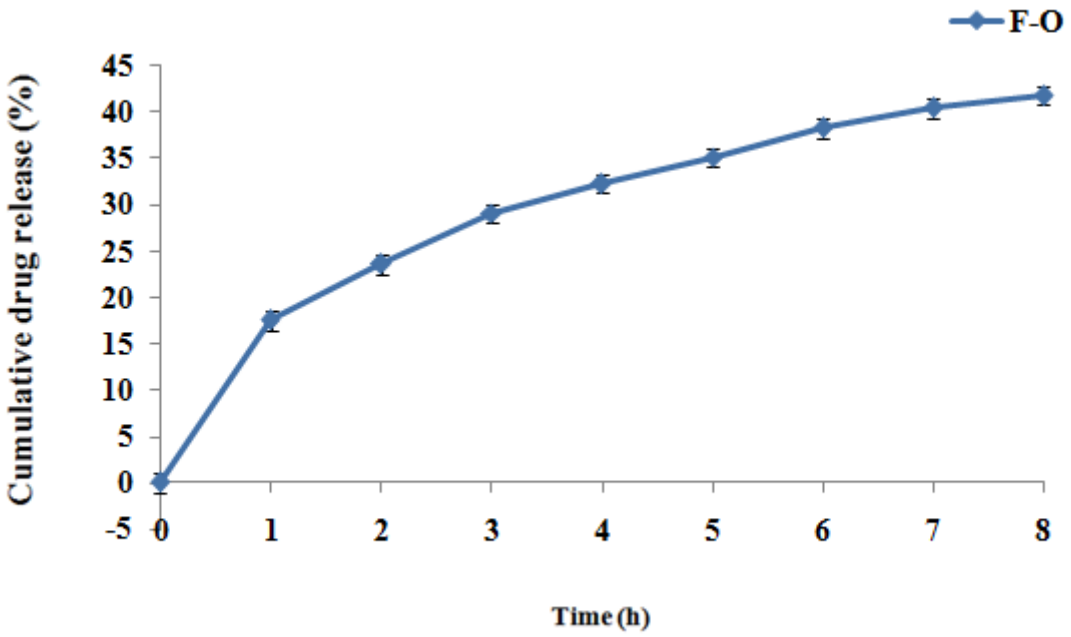

Fig. 12: The *in vitro* cumulative drug release from optimized buoyant beads containing Pioglitazone (F-O) in simulated gastric fluid (pH 1.2)

The release data obtained from in-vitro dissolution study were analyzed kinetically by using several mathematical models like zero order, first order, Higuchi kinetics and Korsmeyar-Peppas model. The result of curve fitting of various mathematical models is shown in **Table 6**. When the respective correlation coefficients (R^2) were compared, it was found that F-1,F-2,F-6, F-8 and F-O were well correlated with Korsmeyar-Peppas model withR^2values of 0.995,0.995,0.992, 0.998, and 0.997. F-5 was correlated with first order kinetics withR^2 values of 0.997 and F-3, F-4 and F-7 were correlated Higuchi kinetics with R^2 values of 0.994, 0.997 and 0.998. The value of diffusion exponent(n) of all buoyant beads containing Pioglitazone was found within the range of 0.385 to 0.490 which affirmed that the release mechanism was Fickian diffusion. This could be further demonstrated the diffusion mechanism in drug release was observed here due to less swelling of pectin in dissolution medium.

Conclusion:

In the current work, formulation optimization of multiple-unit Pioglitazone-loaded buoyant beads was using various polymers like low methoxyl pectin (methoxyl content less than 7%), Poloxamer 407 and magnesium stearate as low density materials as independent variables and their effects on drug entrapment efficiency (DEE, %) and cumulative drug release after 8 h (R_{8h}, %) as responses were analyzed after performing a full 2^3 factorial design. The optimized formula for the preparation of buoyant beads containing Pioglitazone was found 749.98 mg low methoxyl pectin, 345.43 mg Poloxamer 407 and 166.33 mg magnesium stearate. The average diameter, density values, and DEE (%) of these dried beads containing Pioglitazone ranged from 1.67 ± 0.05 to 1.86 ± 0.07 mm, 0.901 ± 0.08 to 0.984 ± 0.09 gm/cm^3 and 66.21 ± 2.72 to 75.43 ± 2.15%. FTIR spectroscopy result suggested the absence of any interaction in-between the drug (here Pioglitazone) and the excipients (namely pectin, Poloxamer 407 and magnesium stearate) in these low-density beads containing Pioglitazone. These formulated beads containing Pioglitazone were showed buoyancy as well as sustained release over 8 h, *in vitro*. These developed Pioglitazone-loaded buoyant beads can be used for gastro-retentive delivery of Pioglitazone in the treatment of type II diabetic mellitus. Other drugs requiring gastro-retentive delivery can be loaded within these buoyant beads.

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