Research Article

Preparation, Characterization and Optimization of Pioglitazone Loaded Microspheres Based Oral Suspension Using 3^2 Factorial Design

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ABSTRACT

The aim of the current research study is to prepare sustained-release Pioglitazone Hydrochloride microspheres. Pioglitazone Hydrochloride microspheres were prepared by Ionotropic gelation method using calcium chloride and sodium alginate. The drug release pattern of developed microspheres was observed. The prepared Microspheres were evaluated for compatibility study, drug entrapment efficiency, In vitro dissolution study, scanning electron microscopy, optical microscopy, differential scanning calorimetry, FTIR. The prepared microspheres show mean particle size of 413.37- 812.37 µm, production yield of 57.13- 80.17%, entrapment efficiency of 42.65- 72.11%, sufficient initial release of 7%, and release of 99.12% of its drug content in 12 hours. Besides that, the surface morphology of microspheres was found to be spherical with smooth surface. Among all the prepared formulations F6 was found to show better satisfactory results. This prepared sustained release Pioglitazone HCl microspheres could enhance the bioavailability of drug thereby improves the patient compliance and expect better treatment than conventional dosage forms.

Keywords: Microspheres, Pioglitazone Hydrochloride, calcium chloride cross-linking method, Ionotropic gelation method.

INTRODUCTION

Oral drug delivery system is the most preferred and attractive route of drug administration due to its ease of administration, stability of formulation and improved[1] patient compliance. The use of controlled drug delivery systems has certain advantages[2] compared with conventional dosage forms, as they can minimize side effects, assures better patient compliance by reducing the frequency of administration of the drug and prolong the efficacy of the drug by maintaining the constant level of drug in blood and tissue[3]. There are a lot of systems developed for sustained or controlled drug release and amongst those, the prominent systems are matrix systems, floating systems and microspheres. Microspheres are the colloidal drug delivery system and sometimes referred to as micro particles. Microspheres are small spherical particles and characteristically free-flowing powders consisting of proteins/synthetic polymers that are biodegradable[4] in nature. Microspheres ideally having the diameter in the micrometer range typically 1 µm to 1000 µm (1 mm).

Diabetes mellitus is a group of syndromes and a chronic metabolic disorder characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins because of a lack of or ineffective use of the hormone insulin. Pioglitazone hydrochloride is an oral antihyperglycemic agent used in the treatment of type 2 diabetes (non-insulin dependent diabetes mellitus). Pioglitazone is indicated as second or third line treatment of type 2 diabetes mellitus. It acts primarily by decreasing insulin resistance. Pioglitazone Hydrochloride is a water insoluble drug with a short biological half-life of 3-6 hrs and is eliminated[5] rapidly from the body. Hence, the present study was aimed to develop the sustained release microspheres of by ionic gelation method using calcium chloride and sodium alginate

MATERIALS AND METHODS

MATERIALS

Pioglitazone hydrochloride (Drug) was obtained from Dr. Reddy's Laboratories, Hyderabad, India. Sodium Alginate was procured from Krishna Pectins Pvt. Ltd. Calcium chloride was purchased from Cognis GMBH and Co., Germany. Glutaraldehyde was obtained from Evonik industries, Mumbai, India. Disodium hydrogen Phosphate was purchased from SD Fine Chem Ltd., Mumbai, India. Acetone and Methanol were purchased from Rankem Fine Chemicals Ltd., New Delhi, India

METHODS

Analytical studies

Determination of absorption maxima values (λmax) using UV-Visible spectrophotometer

Standard stock solution of Pioglitazone hydrochloride (100µg/ml) was prepared in phosphate buffer pH 7.4. For the selection of analytical wavelength, solution of Pioglitazone of concentration 20µg/ml was prepared by appropriate dilution of standard stock solution with phosphate buffer pH 7.4 and scanned in the spectrum range from 200 to 400nm.From the overlain spectrum of the drug, wavelength 269 nm was selected for analysis.

The wavelength with maximum absorption was chosen for further analysis

Preparation of Standard graph of Pioglitazone in pH 7.4 phosphate buffer

The stock solution was freshly prepared by dissolving 100 mg of Pioglitazone in phosphate buffers pH 7.4 in a 100 ml volumetric flask and then making up the solution up to the mark using phosphate buffer pH 7.4 for obtaining the solution of strength 1000 µg/ml (stock I). From this primary stock 10 ml of this solution is diluted to 100ml with phosphate buffer to obtain a solution of strength 100 µg/ml (stock II). From this secondary stock 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 ml were taken separately and made up to 10 ml with phosphate buffer pH 7.4, to produce 10, 20, 30, 40, 50, 60, 70 and 80µg/ml respectively. The absorbance was measured at 269 nm using a UV-Visible spectrophotometer and graph was plotted against the concentration and absorbance.

Drug - excipients compatibility studies

Differential Scanning Calorimetry (DSC)

The physicochemical compatibilities of the drug and the used excipients were tested by DSC analysis. DSC analysis of the pure drug, polymers and the drug-polymer mixture were performed by using an automatic thermal analyzer system (Shimadzu DSC-60) to evaluate the drug-polymer interactions. The analysis was performed at a rate of 10 °C per min from 40 °C to 300 °C under a nitrogen flow of 20 ml/min.

Fourier transform infrared (FTIR) spectroscopy studies[6]

FTIR has been used to study the physical and chemical interactions between drug and the excipients used. KBr pellet is used to know drug-excipient interactions. The samples were scanned over a range of 400-4000 cm^{-1} using FTIR instrument (Alpha, Brucker pvt ltd, Japan).

Experimental Design[7]

Two independent factors, the concentration of Sodium Alginate (X1) and the concentration of Calcium chloride (X2) were set at three different levels. High, middle and low levels of each factor were coded as +1 and -1, respectively and the mean value as zero. The range of a factor must be chosen in order to adequately measure its effects on the response variables. The range of each factor was chosen from the preliminary studies. This design was selected as it provides sufficient degrees of freedom to resolve the main effects as well as the factor interactions. Stepwise regression analysis was used to find out the control factors that significantly affect response variables.

Preparation of Pioglitazone Microspheres

Pioglitazone hydrochloride microspheres were developed by using Ionotropic gelation method, using sodium alginate as polymer and calcium chloride as cross linking agent. Ionotropic gelation is a method to prepare microspheres using combination of Ca^{2+} as cationic components and alginate as anion.

Table 1: Two independent factors

Coded Value	Actual value	
	X_1 (mg)	X_2(%)
-1	100	2
0	150	4
+1	200	6

In this method 5% w/v sodium alginate was prepared dispersing the weighed quantity of sodium alginate with deionized water and stirred for specified time using a magnetic stirrer. Add accurately weighed quantity of pioglitazone hydrochloride, which was dissolved in methanol to sodium alginate solution to get a homogenous drug-polymer mixture. For the formation of microspheres the drug- polymer solution was extruded drop wise from a needle of 18 G in diameter from a height of about 6 cm into 100 ml aqueous calcium chloride solution and stirred at 100 rpm. Then the solution containing microspheres was filtered by using Whatman filter paper. The added droplets were kept dispersed in the calcium chloride solution for 15minutes to complete the curing reactions and to produce spherical rigid microspheres. The microspheres were collected by decantation, and the product, thus separated, was washed repeatedly with water. The microspheres were allowed to dry at about 30^0C- 40^0C for 12- 48 hrs and stored in well closed container for further use.

Table 2: Coded 3^2 Factorial Design for Pioglitazone microsphere

Formulation code	X_2	X_1
F1	+1	-1
F2	+1	0
F3	+1	+1
F4	0	-1
F5	0	0
F6	0	+1
F7	-1	-1
F7	-1	-1
F7	-1	-1

Evaluation of Microspheres

Particle Size and Shape Analysis

Microspheres were examined with respect to their size and shape using optical microscope[8]. Edmondson's equation was used to determine average particle size.

Drug Entrapment Efficiency

100 mg accurately weighted microspheres were cleaned and crushed and dissolved in 100 ml with phosphate

buffer pH 7.4 solution. The microspheres soaked for overnight, then solution was clarified through 0.45μm membrane filter. The solution was analysed for drug content spectrophotometrically at 269 nm with suitable dilution[9].

$$\text{Entrapment efficiency} = \frac{\text{Estimated \% drug content in microspheres}}{\text{Theoretical \% drug content in microspheres}} \times 100$$

Percentage Yield Value

The percentage yield [10] value can be calculated using following equation.

$$\text{Percentage yield} = \frac{\text{Weight of microspheres}}{\text{Total weight of drug and polymer taken}} \times 100$$

In vitro drug release study

A USP paddle apparatus has been used to study *in vitro* drug release from microspheres. *In vitro* drug release studies were carried out for all batches in USP type II dissolution test apparatus[11]at 100 rpm and the dissolution medium used is 900 ml of 0.1 N HCL initially for 2 hrs and phosphate buffer pH 7.4. Microspheres containing 500 mg of drug was used for dissolution study. Five ml of the sample was withdrawn at predetermined intervals upto 12 hrs. Equal volume of the buffer was replaced in the vessel after each withdrawal to maintain sink condition**.** The required dilutions were made with phosphate buffer pH 7.4 and filter the solution and analyzed for the drug content spetrophotometrically at 269 nm.

Kinetic modelling of drug release data

There are a number of kinetic models, which described the overall release of drug from the dosage forms[12]. Data obtained from the *in vitro* release was fit into different equations and kinetic models to explain the release kinetics of Pioglitazone hydrochloride from these microspheres. The kinetic models used were a zero-order equation, first order equation, Higuchi and Korsmeyer-Peppas model. Higuchi model is the most widely used model to describe drug release from pharmaceutical matrices system[13]. The Peppas model is widely used, when the release mechanism is not well known and when more than one type of release is involved. To find out the mechanism of drug release, drug release data were fitted in Korsmeyer-Peppas model[14]. To study the diffusional release mechanism, data obtained from *in vitro* drug release studies were plotted against log cumulative percentage drug release versus log time.

Scanning Electron Microscopy

The shape and surface morphology of the microspheres were examined using scanning electron microscopy[15]. Microspheres were dusted onto double-sided carbon dust, which was placed onto a sample carrier in the shape of cylinder. After fixing the samples on the stubs, capture a photomicrograph.

RESULTS AND DISCUSSION

Determination of absorption maximum values

An UV- Spectrophotometric method was used for estimation of Pioglitazone. The λ_{max} of Pioglitazone (20 µg/ml) in 7.4 pH phosphate buffer was scanned in UV-Visible Spectrophotometer in the wavelength range of 200-400 nm and found to have maximum absorbance at 269 nm.

Preparation of Standard calibration graph of Pioglitazone in pH 7.4 phosphate buffer

Different concentrations of Pioglitazone were prepared in phosphate buffer pH 7.4 (10- 80µg/ml) and absorbance was measured at 269nm. The calibration curve showed a good linearity bearing equation y = 0.012x-0.001 with correlation coefficient of R^2 0.999

Drug - excipient compatibility studies

Differential Scanning Calorimetry (DSC)

From the DSC study it was observed that there was no significant drug polymer interaction observed among drug, Sodium Alginate, calcium chloride even at higher temperature. From DSC study, it is observed that there is no change in drug's melting peak (169.28^{0}C–172.77 ^{0}C) after the preparation of mixture. Hence it can be concluded that drug is compatible with all polymers.

Fourier transform infrared spectroscopy (FTIR)

From the FTIR study it was observed that the drug exhibits peaks due to amide group, alcohol group and C-H, Ar-O-CH, C=C and C-O-C stretching. It was observed that there were no significant changes in drug and mixture excipients. The FTIR study revealed that drug and excipients were compatible.

Formulation of the optimized formula

Pioglitazone microspheres have three different variables include: sodium alginate (X1) polymer, calcium chloride (X2) and a ratio of Drug(X3) were screened using 3 factorial design and nine different formulae of pioglitazone microspheres. Hence Pioglitazone was chosen was as a model drug with an aim to develop a sustained release system for 12 hrs. 3^2 Factorial design was applied to optimize the formulation using sodium alginate (X1) and Calcium chloride (X2) as independent variable. The Responses are Drug release and Entrapment efficiency, the entrapment efficiency is increased with increased concentration of sodium alginate, the particle size and drug release is affected by Calcium chloride concentration. From the all batches F6 is considered the best formulation, because among all other formulations, it shows better extent of drug.

Evaluation parameters

Sodium alginate microspheres of Pioglitazone were successfully prepared by Ionization gelation technique. The results indicate that optimum concentration of Sodium alginate (150mg) and Calcium chloride (6%) showed higher percent of entrapment efficiency (F6) and the values found in range of 42.65% to 72.11%. The percentage yield of microspheres was between 57.13 %.to 80.17, while the size of prepared microspheres in this study was within range of 413.37µm to 812.37 µm. It was observed that as the amount of polymer increases in the microspheres, the particle size also proportionally increased. The results were shown in table 3.

Table 3: Characterization of Microspheres of different batches

Formulation code	Entrapment efficiency (%)	Percentage of yield (%)	Average particle size (µm)
F1	51.18±0.03	67.21	782.67
F2	50.18±0.13	63.17	794.11
F3	48.11±0.13	57.13	678.11
F4	60.03±0.01	70.11	734.21
F5	68.76±0.03	78.11	519.47
F6	72.11±0.13	80.17	812.37
F7	54.65±0.07	72.13	413.37
F8	52.11±0.01	61.17	512.37
F9	42.65±0.11	57.53	612.27

In vitro drug release studies

Drug release studies were carried out by buffer change method to mimic the GIT environment. Initially it was carried out in 0.1 N HCL for 2hrs, the drug release in all formulations was found to be low ranging from 5.14 to 24.13 %. After 2hrs, then drug release studies were performed in phosphate buffer pH 7.4 upto 12hrs. The drug release pattern from all the formulations was sustained for 12 hrs. F1 to F5 Showed the fast release of the drug and Entrapment efficiency was found to good. , In case of formulation F5 the rate of drug release was much faster and found to be 99.87% in 10hrs and drug release was faster rate than the other formulations in 12hrs.But in F6 formulation the drug release was found to be very good and satisfactory. As the concentration of polymer increases, the dissolution rate is decreased, and percent release was not satisfactory in F7, F8 and F9. Finally on the basis of drug release, F6 formulation considered as optimized formulation with maximum 99.12% release of Pioglitazone hydrochloride. The drug release results were shown in figure 1

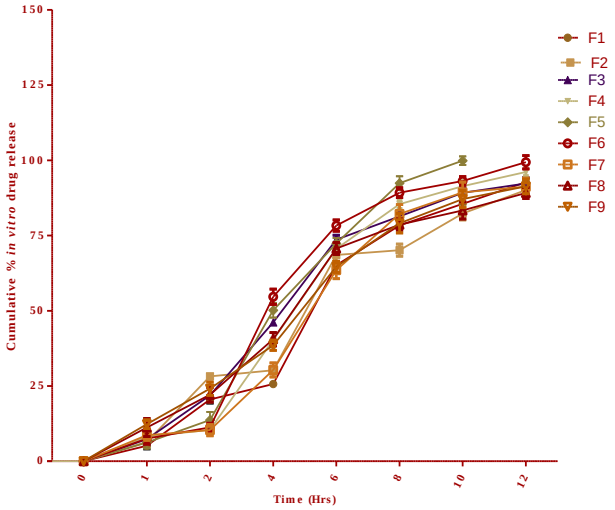

Figure 1: Cumulative percentage of drug release from all formulation

Release kinetics of drug release data

The release data of Pioglitazone were fitted to various equations of release kinetics. Most formulations were fit better with Higuchi model (R^2=0.8991–0.9954) and Korsmeyer–Peppas (R^2=0.8351–0.9958) equations than other equations (zero order, first order). Moreover, the values of $R^2$for the rest of the formulations that fit the first order were very close to the Higuchi model that explains the diffusion control kinetic.

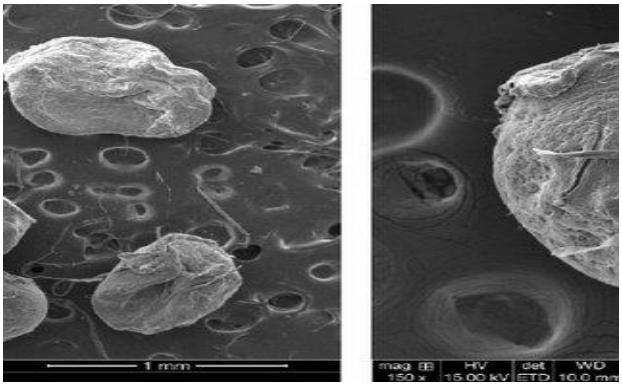

Figure 2: SEM photograph of optimised Pioglitazone hydrochloride (F6)

SEM analysis

SEM analysis revealed that all prepared microspheres were spherical in shape, non aggregated with rough and porous surface, as shown in scanning electron micrographs in figure 2.

Experimental design

Based on the preliminary experiments and our previous studies, two factors (sodium alginate and calcium chloride) were identified as key factors responsible for % entrapment efficiency and % drug release of microspheres.[7]

Table 4: Regression coefficient (R^2) values of Pioglitazone microspheres for different kinetic models

Formulation	Zero order (R^2)	First order (R^2)	Higuchi R^2	Peppas R^2	N
F1	0.9647	0.9337	0.9350	0.9351	1.66
F2	0.9635	0.9647	0.9251	0.9354	1.65
F3	0.9247	0.9647	0.9457	0.9958	1.66
F4	0.9143	0.9647	0.9751	0.9351	1.62
F5	0.8746	0.9647	0.9354	0.9364	0.53
F6	0.9835	0.9647	0.9954	0.9351	0.43
F7	0.9641	0.9647	0.9351	0.8351	0.99
F8	0.9642	0.9647	0.9261	0.9351	1.32
F9	0.9543	0.9647	0.8991	0.8351	1.43

The polymer sodium alginate was used because it decreases the drug release from the microsphere at optimum concentration. The relationship between the dependent and independent variables is further elucidated by constructing the response surface plot and counter plot. The three-dimensional (3D) response surface graphs generated by the design expert software (trial versions version 9.0.1) for the most statistical significant variables on the evaluated parameters are presented in Figures 3‑4. The 3D response surface curves are used for studying the interaction patterns. On the basis of 3D response surface graphs, it can be said that the sodium alginate concentration and crossing linking agent calcium chloride produces a significant effect on entrapment efficiency and drug release percentage [Figures 8, 10 and 12]. The quadratic response surface was studied for amount of drug release which helped in knowing the interaction effects between the selected independent variable & for Cumulative Drug Release. The variation in drug release and entrapment efficiency was observed on changing the concentration of polymer and cross linking agent. The F6 exhibited sustained drug release at slower rate upto 12hrs and highest drug entrapment efficiency compared to other formulations. Hence, from experimental design F6 formulation considered as optimized formulation.

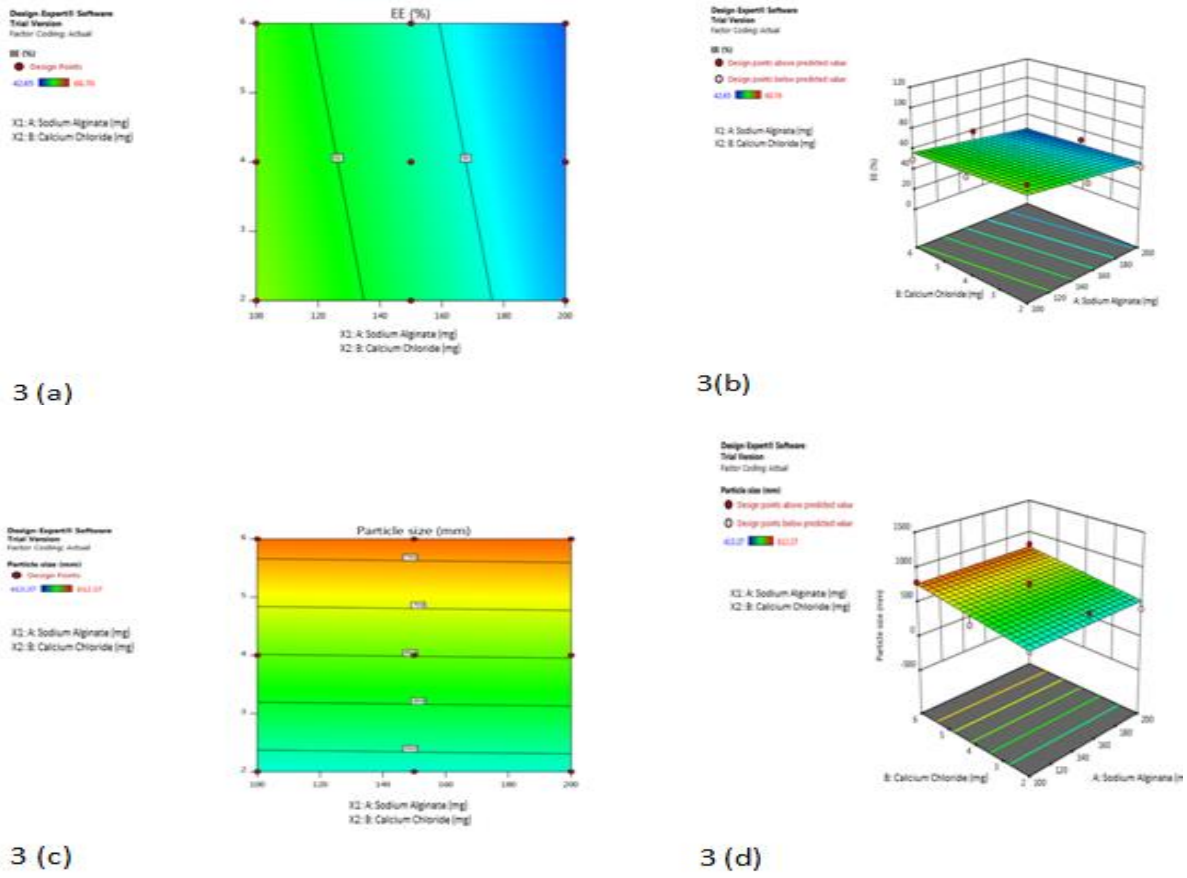

Figure 3: (a) Counter plot for Entrapment efficiency, (b) 3D plot for entrapment efficiency, (c) Counter plot for particle size analysis, and (d) 3D plot for particle size analysis

CONCLUSION

The microspheres of Pioglitazone hydrochloride were prepared with different concentration of sodium alginate in different batches. From the developed formulation (F6) of Pioglitazone containing Sodium alginate (150 mg), Calcium chloride (6%) show excellent results with maximum drug release of 99.12%. It was concluded that microspheres of Pioglitazone were optimized using three-level, two factorial experimental design. The formulation variable sodium alginate and variable Cross linking Calcium chloride exerted a significant influence on the drug entrapment and drug release. The findings acquired suggested that response surface methodology can be successfully used to evaluate the effect of formulation variables and improve an optimized formulation thereby reducing the number of trials, time, and cost of formulation development. Hence, prepared microspheres may prove to be potential candidates to enhance the bioavailability in the upper part of the gastrointestinal tract and improve patient compliance.

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