Formulation, Characterization And Optimization Of Pioglitazone Hydrochloride Nanoparticles By Solvent Displacement Method Using 3² Factorial Design

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Abstract: The aim of the present work was to formulate nanoparticles for pioglitazone hydrochloride drug. Pioglitazone hydrochloride is a antidiabetic drug, and BCS Class – II drug having low solubility and high permeability. Nanoparticles were prepared by Solvent displacement method using 3² full factorial design. The concentration of Chitosan (X₁) and Pluronic F68 (X₂) were chosen as independent variables while percentage drug release at 12th hour, drug entrapment efficiency and particle size was taken as dependent variables. The dissolution profile of all nine factorial formulations was fitted to zero order, first order, Higuchi and Korsemayer Peppas models to ascertain the kinetic modeling of drug release. The prepared formulations were further evaluated for drug content, drug excipient interactions, surface morphology by SEM, Differential scanning calorimetry (DSC), Zetapotential. All independent variables were found to significantly influence the particle size and entrapment efficiency. The in-vitro drug release profile showed that the suitability of Chitosan loaded nanoparticles in sustaining pioglitazone release for prolonged time.

Key words: Pioglitazone, Chitosan, Solvent Displacement method, 3² full factorial designs.

INTRODUCTION
Diabetes is a serious metabolic disorder with micro and macrovascular complications that results in significant morbidity and mortality. The most common complications of diabetes are cardiovascular disease and stroke. According to WHO the number of diabetics will rise from 15 million in 1995 to 57 million by 2025 making it the country with the highest number of diabetics in the world. Diabetes is the sixth leading cause of death in the US, according to the center for disease control and prevention (CDC). This accounts to 224,000 death in year 2002 only. A report says that diabetic is responsible for 109,000 death in 2004 approximately 157 million years of life lost in 2004 nearly 2.263 million disability adjusted life years (DALYs) in India during 2004.

Majority of the antidiabetic drugs including Pioglitazone hydrochloride belongs to class-II of BCS. It is an oral antidiabetic agent belonging to the thiazolidinedione (TZD) class of medications. It is a water insoluble drug with short half life of 3-7 years and is eliminated rapidly from the body. Pioglitazone selectively stimulates the nuclear receptor peroxisome proliferator- activated receptor gamma (PPARγ) and to a lesser extent PPAR-α. Pioglitazone hydrochloride is used for the treatment of diabetes mellitus type 2 in monotherapy and in combination with a sulfonylurea, metformin, orr insulin. Pioglitazone also lowers the level of glucose in the blood by reducing the production and secretion of glucose into the blood by the liver. The low solubility in aqueous condition limits the enhancement of pharmacokinetics and bioavailability of the drug.
Nanoparticles may be used for oral administration of gut-labile drugs or those with low aqueous solubility. These colloidal carriers have the ability to cross the mucosal barrier as such. In addition to the potential for enhancing drug bioavailability via particle uptake mechanisms; nanoparticulate oral delivery systems also have slower transit times than larger dosage forms increasing the local concentration gradient across absorptive cells, thereby enhancing local and systemic delivery of both free and bound drugs across the gut. These colloidal carriers are expected to develop adhesive interactions within the mucosa and remain in the gastrointestinal tract, while protecting the entrapped drug from enzymatic degradation until the release of the loaded drug or their absorption in an intact particulate form.

The proper selection of the polymeric matrix is necessary in order to develop a successful nanoparticulate delivery system. Biodegradable polymers have received much attention in recent years. Chitosan has been most extensively used because of its biocompatibility, biodegradability and low toxicity. It is a natural linear polyamine with a high rate of glucosamine to acetyl-glucosamine units, is a weak base and carries a positive charge. Its solubility is pH dependent and it reacts readily with negatively charged surfaces and materials including polymers and DNA.

This article reports the design of biodegradable nanoparticles containing pioglitazone hydrochloride for oral delivery.

In the present investigation chitosan was used as polymers and pluronic F68 used as stabilizer. Nanoparticles were prepared by solvent displacement method. From the preliminary trials, the constraints for independent variables $X_1$ (Amount of chitosan) and $X_2$ (Amount of Pluronic F68) have been fixed. The polynomial equations for particle size, percentage drug entrapment and % drug release were derived. The prepared formulations were further evaluated for drug content, in vitro drug release pattern, and drug excipient interactions. The application of factorial design gave a statistically systematic approach for the formulation and optimization of nanoparticles with desired particle size and high entrapment efficiency and percentage release.

**MATERIALS AND METHODS**

**Materials**

Pioglitazone hydrochloride was obtained as gift sample from Actavis pharma, Chitosan was obtained as gift sample from Actavis Pharma, Pluronic F68 was purchased from S.D Fine chemicals, dialysis bag (cellophane membrane, molecular weight cut off 10000-12000 Da) purchased from Hi media, Mumbai. India, all other reagents and chemicals used in this study were of analytical grade.

**METHODS**

**Full factorial design**

A $3^2$ full factorial design was used in the present study. In this design two factors were evaluated, each at 3 level and experimental trials were performed of all nine possible combinations. The concentration of Chitosan ($X_1$) and the Pluronic F68 ($X_2$) were chosen as independent variables in $3^2$ full factorial design, particle size, entrapment efficiency and percentage drug release at 12th hour were taken as dependent variables. A statistical model incorporating interactive and polynomial terms was used to evaluate the responses. The formulation layout for the factorial design batches (F1-F9) is shown in table No.1

**Preparation of Pioglitazone hydrochloride loaded Nanoparticles**

Pioglitazone nanoparticles were prepared by Solvent displacement method. An accurately weighed amount of Pioglitazone hydrochloride (150mg) was dissolved in 9ml mixture of Acetone and Methanol (2:1) using sonication for 5 minutes. Specified amounts of Chitosan were dispersed in aqueous surfactant solution. The organic phase was poured slowly into 30ml of aqueous surfactant solution with stirring and 70ml of water was added subsequently with magnetic stirring at 1000rpm for 60 minutes. The aqueous surfactant solutions contains a non ionic surfactant (Pluronic F68) at various concentrations (1, 1.5, 2% w/v) Nanoparticles were immediately formed and organic solvents were then removed from the colloidal suspension by evaporation using rotavapor. Various colloidal formulations were purified from unincorporated drug and unabsorbed non ionic surfactants by means of centrifugation and pellets were washed with water.
Characterization of Pioglitazone Loaded Chitosan Nanoparticles

Measurement of Particle size and Zeta potential:
The particle size and size distribution of the pioglitazone hydrochloride loaded chitosan nanoparticles were determined by photon correlation spectroscopy using a Zetasizer 2000 Malvern Instruments, U.K. Nanosuspensions were diluted with filtered (0.22µm) ultra pure water and analyzed using Zetasizer, yielding the mean particle diameter of the suspension (Z – average, measuring range: 20- 1000 nm) and Polydispersity index (PDI)13.

Electrophoretic mobility was obtained by a laser Doppler anemometer using the same instrument. A suitable amount of the sample (50-100µl) was diluted with 5ml of water (HPLC grade) and placed into the electrophoretic cell of the instrument, where a potential of ±150mV was induced. The zeta potential value was calculated by the software using Smoluchowski’s equation.

Entrapment efficiency (EE):
The encapsulation efficiency of nanoparticles was determined by the separation of drug-loaded nanoparticles from the aqueous medium containing non-associated pioglitazone14. The entrapped drug was determined by taking 2ml of nanoparticle suspension and centrifuged by ultracentrifugation (REMI high speed, cooling centrifuge, REMI Corporation, India) at 15000 rpm at 4°c for 30 minutes. The amount of pioglitazone hydrochloride loaded into the nanoparticles was calculated as the difference between the total amount used to prepare the nanoparticles and the amount that was found in the supernatant. The amount of free pioglitazone in the supernatant was measured at 270nm using UV-visible Spectrophotometer (Shimadzu UV-1700) after suitable dilution with 0.1N HCl. The pioglitazone hydrochloride encapsulation efficiency (EE) of the nanoparticles was determined in triplicate and calculated as follows–

\[
\text{Percentage drug entrapment efficiency} = \frac{W_{\text{initial drug}} - W_{\text{free drug}}}{W_{\text{initial drug}}} \times 100
\]

Statistical Analysis:
The results from factorial design were evaluated using PCP Disso 2000 V3 software. Stepwise backward lineintceclear regression analysis was used to develop polynomial equations for dependent variables particle size (Y1), % drug entrapment (Y2) and % drug release at 12th hour (Y3) Which bear the form of equation-1.

\[
Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 \ldots \ldots 1
\]

Where Y is dependent variable, b0 arithmetic mean response of nine formulations, and b1 estimated coefficient for factor X1. The main effects (X1 and X2) represent average result of changing one factor at a time from its low to high value. The interaction term (X1X2) shows how the response changes when two factors are simultaneously changed. The polynomial terms (X1^2 and X2^2) are included to investigate non-linearity12.

Scanning Electron Microscopy (SEM) Analysis:
The morphology and size of statistically optimized formulation of pioglitazone hydrochloride loaded nanoparticles were examined by scanning electron microscopy (SEM, Hitachi S3400N, Japan)15. The nanoparticles were mounted on metal stubs using double-sided tape and coated with a 150 A° layer of gold under vacuum. Stubs were visualized under scanning electron microscope.

Fourier Transform Infra Red Spectroscopy (FTIR)
The FTIR spectra of pioglitazone hydrochloride loaded nanoparticles were determined by using Shimadzu S4008 model. The pellets were prepared by gently mixing of 1mg sample with 200mg potassium bromide at high compaction. A base line correction was made using dried potassium bromide and the spectra of dried nanoparticles were recorded. Thus the prepared pellet was scanned over the range of 4000 cm⁻¹ to 400 cm⁻¹ with a resolution of 4cm⁻¹ for 50 scans.
Differential Scanning Calorimetry Analysis (DSC):

Differential scanning calorimetry (DSC) are one of the most powerful analysis technique which offering the possible of detecting chemical interaction between drug and polymer. The differential scanning calorimetry of thermograms of lyophilized pioglitazone loaded nanoparticle suspensions were obtained using an automatic thermal analyzer system (Pyris 6 DSC, Q20V24.4 Build 1.16, Perkin-Elmer, USA). Temperature calibration was performed using indium calibration reference standard (transition point: 150.60°C) as a standard. Samples were crimped in standard aluminum pans and heated from 40 to 400°C at a heating rate of 10°C/min under constant purging of dry nitrogen at 30ml/min. An empty pan sealed in the same way as the sample, was used as a reference.

Drug Release from Nanoparticles:

The in vitro drug release studies were performed using the dialysis membrane diffusion technique. The dialysis membrane of 12000Mwt cut off was used. The membrane was soaked before use in distilled water for 4 hours then rinsed thoroughly in distilled water. One ml of pioglitazone hydrochloride nanoparticles dispersions, equivalent to (1.5mg/ml) of pioglitazone was transferred in to dialysis membrane bag, tied and placed in beaker containing 100ml of Dissolution medium. The entire system was kept at 37°C±0.5°C with continuous magnetic stirring (50rpm) and the study was carried out in two dissolution media; 0.1N HCl pH 1.2, and Phosphate buffered saline (PBS) pH 7.4. At appropriate time intervals 5ml of release medium was removed and 5ml fresh medium was added into the system to maintain sink condition. The amount of pioglitazone in the release medium was evaluated by U.V Spectrophotometer at 270nm.

RESULTS AND DISCUSSION:

All the factorial formulations developed by the emulsification solvent evaporation method, formulations were found to be free flowing, white and powdery in appearance.

Particle size and Entrapment Efficiency:

Particle size is an important parameter because it has a direct relevance to the stability of the formulation. Larger particles tend to aggregate to a greater extent compared to smaller particles, thereby resulting in sedimentation. The amount of stabilizer used also has an effect on the properties of nanoparticles. If the concentration of stabilizer is too low, aggregation of the polymer will take place, whereas, if too much stabilizer is used, drug incorporation could be reduced as a result of the interaction between the drug and stabilizer.

From fig.1 and 2 and table 1, it is revealed that as drug: polymer ratio increased from 1:1 to 1:2 particle size increased significantly and drug entrapment also increased but thereafter, further increase in drug:polymer ratio showed reduced in the drug entrapment efficiency.

This can be explained by observing drug entrapment efficiency of factorial formulations F1, F2, F3 where drug:polymer ratio increased from 1:1, 1:2, and 1:3 respectively with constant concentration of stabilizer (Pluronic F 68) i.e.1%. Drug entrapment efficiency increased from 64.81% to 69.29 and then decreased it to 67.64%. It is also observed that as percentage of stabilizer increased from 1% to 2% entrapment efficiency increased and particle size decreased significantly. Thus it can be concluded that the stabilizer had greater influence on both dependent parameters (particle size and entrapment efficiency).
Table 1: Experimental design and parameters for $3^2$ Full Factorial Design batches

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>Variable levels in Coded Form</th>
<th>Particle Size (nm)</th>
<th>% Drug Entrapment ± SD*</th>
<th>% Drug Release at 12th hour ± SD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>-1 -1</td>
<td>345.8</td>
<td>64.81±0.61</td>
<td>58.61±0.41</td>
</tr>
<tr>
<td>F2</td>
<td>0 -1</td>
<td>608.6</td>
<td>69.29±0.47</td>
<td>53.24±0.04</td>
</tr>
<tr>
<td>F3</td>
<td>+1 -1</td>
<td>646.6</td>
<td>67.64±0.57</td>
<td>48.55±0.33</td>
</tr>
<tr>
<td>F4</td>
<td>-1 0</td>
<td>285.6</td>
<td>68.44±0.59</td>
<td>64.32±0.48</td>
</tr>
<tr>
<td>F5</td>
<td>0 0</td>
<td>479.9</td>
<td>72.5±0.44</td>
<td>59.06±0.13</td>
</tr>
<tr>
<td>F6</td>
<td>+1 0</td>
<td>531.2</td>
<td>69.48±0.68</td>
<td>54.93±0.42</td>
</tr>
<tr>
<td>F7</td>
<td>-1 +1</td>
<td>257.3</td>
<td>74.14±0.76</td>
<td>66.21±0.36</td>
</tr>
<tr>
<td>F8</td>
<td>0 +1</td>
<td>351.2</td>
<td>73.79±1.18</td>
<td>59.42±0.06</td>
</tr>
<tr>
<td>F9</td>
<td>+1 +1</td>
<td>406.4</td>
<td>73.48±1.52</td>
<td>55.22±0.09</td>
</tr>
</tbody>
</table>

*All the tests were carried out in triplicate
Table-2 Translation of coded levels to actual quantities

<table>
<thead>
<tr>
<th>Coded Levels</th>
<th>+1</th>
<th>0</th>
<th>-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount of chitosan (X₁)(mg)</td>
<td>150</td>
<td>300</td>
<td>450</td>
</tr>
<tr>
<td>Amount of Pluronic F68(X₂) (%)</td>
<td>1</td>
<td>1.5</td>
<td>2</td>
</tr>
</tbody>
</table>

Differential Scanning Calorimetry Analysis (DSC):

DSC studies were performed to investigate the physical state of the drug in the nanoparticles, because this aspect could influence the *in vitro* and *in vivo* release of the drug from the system. DSC thermogram of pure Pioglitazone hydrochloride shows an endotherm at 192.75°C corresponding to the melting, immediately followed by an exotherm corresponding to the recrystallization of the melt, which then decomposes exothermically at about 255.5°C. The melting peak of pure drug was slightly reduced in the thermogram of loaded nanoparticles evidencing the absence of crystalline drug in the nanoparticle sample. It may be hypothesized that the polymer inhibited the crystallization of pioglitazone hydrochloride during nanoparticles formation. Therefore it could be concluded from the DSC of optimized batch that pioglitazone hydrochloride in the nanoparticles was in an amorphous or a solid solution state in the polymer matrix after the production.

![Figure no-3: DSC Thermogram of Drug (Pioglitazone hydrochloride)](image3)

![Figure no-4: DSC Thermogram of Drug and Polymer](image4)
Fourier Transform Infra Red Spectroscopy (FTIR)

FTIR study was carried out to confirm the compatibility between the selected polymer Chitosan and drug pioglitazone hydrochloride are presented in fig.no 6 and 7. The spectra obtained from the I.R. studies are from 3600cm\(^{-1}\) to 400cm\(^{-1}\). It was confirmed that there are no major shifting as well as no loss of functional peaks between the spectra of drug and polymer.
In vitro drug release:

The *in vitro* release results of pioglitazone loaded chitosan nanoparticles formulations are shown in fig no 8,9 and 10. It can be observed that all runs showed a biphasic drug release, with an initial burst release (15.44% to 20.34%) in the first hour followed by a sustained release for 24 hours. Burst release is characterized by the release of the drug that is incorporated on or near the core interface. This is controlled by diffusion rather than particle degradation. The second phase or the linear release is characterized by pore formation and particle deformation. The drug to polymer ratio was found to affect the drug release from different nanoparticles formulations. It is clear that the effect of drug to polymer ratio on drug release is closely related to the surfactant used. The increase in drug to polymer ratio from 1:1 to 1:2 and 1:3 appeared to substantially decrease the release in all formulations. This was due to the presence of a thicker coat and therefore a longer path to diffuse through. On the other hand, increasing the surfactant concentration from 1% to 1.5% and 2% appeared substantially increase the release in all the formulations. This was due to the formation of smaller particle size and therefore larger surface area.

![Figure no-8: Cumulative % drug release of Formulations F1-F3](image1)

![Figure no-9: Cumulative % drug release of Formulations F4-F6](image2)
Kinetics of drug release: curve fitting and model fitting:

The dissolution profile of all the batches were fitted to various mathematical models for describing the release mechanism for pioglitazone nanoparticles; Kosmeyer-Peppas, Zero order and Higuchi release model to ascertain the kinetic modeling of drug release by using a (PCP Disso V 2.08) software and the model with the higher correlation coefficient was considered to be the best fit model.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Zero order R² value</th>
<th>First order R² value</th>
<th>Higuchi’s R² value</th>
<th>Peppas R² value</th>
<th>n value</th>
<th>Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>0.8490</td>
<td>0.9845</td>
<td>0.9989</td>
<td>0.9972</td>
<td>0.4575</td>
<td>Matrix</td>
</tr>
<tr>
<td>F2</td>
<td>0.8659</td>
<td>0.9811</td>
<td>0.9988</td>
<td>0.9972</td>
<td>0.4606</td>
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<tr>
<td>F3</td>
<td>0.8725</td>
<td>0.9767</td>
<td>0.9984</td>
<td>0.9966</td>
<td>0.4682</td>
<td>Matrix</td>
</tr>
<tr>
<td>F4</td>
<td>0.8200</td>
<td>0.9870</td>
<td>0.9984</td>
<td>0.9978</td>
<td>0.4421</td>
<td>Matrix</td>
</tr>
<tr>
<td>F5</td>
<td>0.8347</td>
<td>0.9827</td>
<td>0.9981</td>
<td>0.9972</td>
<td>0.4366</td>
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<tr>
<td>F6</td>
<td>0.8472</td>
<td>0.9800</td>
<td>0.9984</td>
<td>0.9964</td>
<td>0.4391</td>
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<tr>
<td>F7</td>
<td>0.8485</td>
<td>0.9917</td>
<td>0.9979</td>
<td>0.9958</td>
<td>0.4508</td>
<td>Matrix</td>
</tr>
<tr>
<td>F8</td>
<td>0.8472</td>
<td>0.9856</td>
<td>0.9974</td>
<td>0.9953</td>
<td>0.4450</td>
<td>Matrix</td>
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<tr>
<td>F9</td>
<td>0.8513</td>
<td>0.9791</td>
<td>0.9975</td>
<td>0.9948</td>
<td>0.4397</td>
<td>Matrix</td>
</tr>
</tbody>
</table>

Scanning Electron Microscopy (SEM) Analysis:

The morphology and size of statistically optimized formulation of pioglitazone hydrochloride loaded nanoparticles were examined by scanning electron microscopy (SEM, Hitachi S3400N, Japan). The external morphological study using SEM revealed that all nanoparticles were spherical in shape shown in fig no.11.

Polynomial Equations:

The polynomial equations for three dependent variables (particle Size, %drug entrapment and %drug release at 12th hour) have been derived using PCP Disso 2000 V3 software according the data of experimental design and parameters (Table-1) for factorial formulations F1 to F9.

The equation derived for particle size is:

\[ Y_1 = 434.733 + 115.9167X_1 - 97.6833X_2 \quad \ldots \ldots 2 \]
The equation derived for % drug entrapment is:

\[ Y_2 = 70.3967 + 3.2783 X_2 \quad \ldots \ldots 3 \]

The equation derived for % drug release at 12th hour is:

\[ Y_3 = 59.44 - 5.07 X_1 + 3.4083 X_2 - 2.5617 X_2^2 \quad \ldots \ldots 4 \]

In equations (2) negative sign for coefficient of \( X_2 \) indicates that the particle size of nanoparticles increases when concentration of stabilizer (Pluronic F68) is decreased and positive sign for coefficient of \( X_1 \) indicate positive effect of polymer concentration (Chitosan) on particle size.

In equations (3) positive sign for coefficient of \( X_2 \) indicates that the % drug entrapment increases when concentration of stabilizer (Pluronic F68) increases.

In equation (4) negative sign for coefficient of \( X_1 \) indicates that % drug release of nanoparticles increases when concentration of polymer (Chitosan) decreases and positive sign for coefficient of \( X_2 \) indicates that % drug release of nanoparticles increases when concentration of stabilizer (Pluronic F68) increases.

**Response surface plots:**

Graphical presentation of the data can help to show the relationship between response and independent variables. Graph gave information similar to that of the mathematical equations obtained from statistical analysis. The response surface graph of particle size, % drug entrapment and % drug release at 12th hour were presented in figures-12, 13 and 14 respectively.

The response surface plots illustrated that as concentration of polymer (Chitosan) increases, the value of dependent variable i.e. particle size increases and as concentration of stabilizer (Pluronic F68) increases the value of dependent variable i.e. particle size decreases. Similarly the response surface plots for % drug entrapment shows positive effect of independent variable i.e. stabilizer concentration (Pluronic F68). The response plots for % drug release at 12th hour shows positive effects of independent variable i.e. stabilizer concentration (Pluronic F68) and negative effect of other independent variable i.e. concentration of polymer (Chitosan).

![Figure no-11: SEM Photomicrograph of Pioglitazone loaded Chitosan Nanoparticle (x50,000). Scale bar=100µm](image)
Figure no-12: Response surface plot showing effect of factorial variables on Particle size

Figure no-13: Response surface plot showing effect of factorial variables on % Drug entrapment
CONCLUSION:

Pioglitazone hydrochloride loaded nanoparticles were prepared by the emulsification solvent displacement method. The application of factorial design gave a statistically systematic approach for the formulation of nanoparticles with desired particle size, high entrapment efficiency and % drug release. Drug: Polymer ratio and concentration of stabilizer were found to influence the particle size, Entrapment efficiency and % drug release of pioglitazone hydrochloride loaded chitosan nanoparticles but the concentration of stabilizer had greater influence on dependent variables (Particle size, %drug entrapment and % drug release at 12th hour) as compared to Drug: Polymer ratio. In vitro drug release study of optimized formulation (F7) showed 89.37% release in 24 hours. The release was found to follow matrix release kinetics with Fickian diffusion mechanism for all batches. These results indicate that pioglitazone loaded Chitosan nanoparticles could be effective in sustaining drug release for a prolonged period.

REFERENCES:


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